

# UC Davis

## UC Davis Previously Published Works

### Title

Current trends in drug metabolism and pharmacokinetics.

### Permalink

<https://escholarship.org/uc/item/4qm8f0vf>

### Journal

Acta pharmaceutica Sinica. B, 9(6)

### ISSN

2211-3835

### Authors

Li, Yuhua  
Meng, Qiang  
Yang, Mengbi  
et al.

### Publication Date

2019-11-01

### DOI

10.1016/j.apsb.2019.10.001

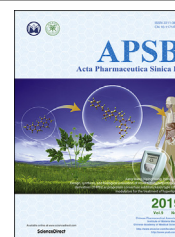
Peer reviewed



Chinese Pharmaceutical Association  
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

[www.elsevier.com/locate/apsb](http://www.elsevier.com/locate/apsb)  
[www.sciencedirect.com](http://www.sciencedirect.com)



## ANNUAL REVIEW

# Current trends in drug metabolism and pharmacokinetics



Yuhua Li<sup>a,b,†</sup>, Qiang Meng<sup>c,†</sup>, Mengbi Yang<sup>d,†</sup>, Dongyang Liu<sup>e,†</sup>,  
Xiangyu Hou<sup>f,†</sup>, Lan Tang<sup>g,†</sup>, Xin Wang<sup>h,†</sup>, Yuanfeng Lyu<sup>d</sup>,  
Xiaoyan Chen<sup>f,\*</sup>, Kexin Liu<sup>c,\*</sup>, Ai-Ming Yu<sup>i,\*</sup>, Zhong Zuo<sup>d,\*</sup>,  
Huichang Bi<sup>a,\*</sup>

<sup>a</sup>School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510275, China

<sup>b</sup>The First Affiliated Hospital of Nanchang University, Nanchang 330006, China

<sup>c</sup>College of Pharmacy, Dalian Medical University, Dalian 116044, China

<sup>d</sup>School of Pharmacy, the Chinese University of Hong Kong, Hong Kong, China

<sup>e</sup>Drug Clinical Trial Center, Peking University Third Hospital, Beijing 100191, China

<sup>f</sup>Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

<sup>g</sup>School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China

<sup>h</sup>School of Life Sciences, East China Normal University, Shanghai 200241, China

<sup>i</sup>UC Davis School of Medicine, Sacramento, CA 95817, USA

Received 7 April 2019; received in revised form 23 August 2019; accepted 9 September 2019

## KEY WORDS

Pharmacokinetics;  
Drug metabolism;  
Drug–drug interactions;  
Modeling;  
Metabolizing enzymes;  
Transporters;  
Nuclear receptors;  
Noncoding RNAs

**Abstract** Pharmacokinetics (PK) is the study of the absorption, distribution, metabolism, and excretion (ADME) processes of a drug. Understanding PK properties is essential for drug development and precision medication. In this review we provided an overview of recent research on PK with focus on the following aspects: (1) an update on drug-metabolizing enzymes and transporters in the determination of PK, as well as advances in xenobiotic receptors and noncoding RNAs (ncRNAs) in the modulation of PK, providing new understanding of the transcriptional and posttranscriptional regulatory mechanisms that result in inter-individual variations in pharmacotherapy; (2) current status and trends in assessing drug–drug interactions, especially interactions between drugs and herbs, between drugs and therapeutic biologics, and microbiota-mediated interactions; (3) advances in understanding the effects of diseases on PK, particularly changes in metabolizing enzymes and transporters with disease progression; (4) trends in mathematical modeling including physiologically-based PK modeling and novel animal models such as CRISPR/Cas9-

\*Corresponding authors. Tel.: +86 20 39943470; fax: +86 20 39943000.

E-mail addresses: [xychen@sim.ac.cn](mailto:xychen@sim.ac.cn) (Xiaoyan Chen), [liukexin89@163.com](mailto:liukexin89@163.com) (Kexin Liu), [aimyu@ucdavis.edu](mailto:aimyu@ucdavis.edu) (Ai-Ming Yu), [joanzuo@cuhk.edu.hk](mailto:joanzuo@cuhk.edu.hk) (Zhong Zuo), [bihchang@mail.sysu.edu.cn](mailto:bihchang@mail.sysu.edu.cn) (Huichang Bi).

<sup>†</sup>These authors made equal contributions to this work.

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

<https://doi.org/10.1016/j.apsb.2019.10.001>

2211-3835 © 2019 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

based animal models for DMPK studies; (5) emerging non-classical xenobiotic metabolic pathways and the involvement of novel metabolic enzymes, especially non-P450s. Existing challenges and perspectives on future directions are discussed, and may stimulate the development of new research models, technologies, and strategies towards the development of better drugs and improved clinical practice.

© 2019 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Pharmacokinetics (PK) is defined as the quantitative study of drug absorption, distribution, metabolism, and excretion (ADME)—*i.e.*, the ways the body processes a drug<sup>1</sup> while the drug exerts its actions in the body. The scope of PK not only covers studies on healthy subjects but also includes broad research on variations under a variety of physiologic or pathologic conditions and the underlying mechanisms, potential drug–drug interactions (DDI), and possible strategies such as dose adjustment to achieve precision medication. Collectively, these aspects of PK allow customization of drug dosage regimens to enhance therapeutic outcomes<sup>1</sup>. Therefore, PK study is a prerequisite to establish the relations and the underlying mechanisms of a drug to its activities and clinical benefits. The information obtained is crucial for lead identification and optimization in drug discovery, as well as dosage regimen design and adjustment in clinical practice<sup>2</sup>. The complexity of PK has evolved, largely in relation to the rapid developments in analytical chemistry, computer science, molecular biology and biochemistry. Although much is known with regard to the PK of many drugs, and many technologies have been established for PK research, recent studies are revealing the existence of new mechanisms by which how drugs are metabolized and how PK is regulated. New experimental models and computational modeling algorithms are arising for an improved understanding of the significance of PK in a whole-body system; nonetheless, many challenges remain.

This review will provide a comprehensive overview of recent developments in the areas of PK research. First, we will provide an update of findings on drug-metabolizing enzymes and transporters in the control of PK, as well as advances in nuclear receptors and noncoding RNAs (ncRNAs) in the modulation of PK, which will provide new insights into understanding the transcriptional and posttranscriptional regulatory mechanisms behind inter-individual variations in pharmacotherapy. Second, we will review the current status and trends in assessing DDIs, especially the interactions between drugs and herbs, between drugs and therapeutic biologics, and microbiota-mediated DDIs and HDIs. Third, we will summarize recent advances in disease–drug interactions, in particular, regulation of metabolizing enzymes and transporters and alteration of PK by different diseases or physiological states. Fourth, we will summarize the trends in mathematical modeling including physiologically-based PK, which could be applied to support clinical investigations. In addition, we will discuss novel animal models such as CRISPR/Cas9-based animal models for DMPK research and overview some interesting non-classical biotransformation pathways including those utilizing novel drug-metabolizing enzymes. Existing challenges and future perspectives are also discussed. It is expected that this review will provide

an update on recent advances in PK fields and may stimulate the establishment of new research models, technologies, and strategies towards the development of better drugs and improvements in clinical practice.

## 2. Determinants of PK

Drug-metabolizing enzymes and transporters play a very important role in the control of PK. Furthermore, transcriptional and posttranscriptional factors such as nuclear receptors and non-coding RNAs (ncRNAs) are critical in the modulation of PK and provide in-depth insight into understanding regulatory mechanisms to solve problems in PK. These mechanism-driven PK studies can improve the success of drug development related to its efficacy and safety and improve the rational use of medication in clinical practice.

### 2.1. Drug-metabolizing enzymes in the control of PK

Drug-metabolizing enzymes mediate the metabolism of exogenous and endogenous substances. Most drugs lose their pharmacological activities mainly through metabolic transformation, yielding metabolites with high water solubility that are readily excreted. Hence, metabolizing enzymes play an extremely important role in the control of drug PK. The biotransformation of xenobiotics by xenobiotic-metabolizing enzymes (XMEs) may be classified into Phase I and Phase II reactions. Advanced characterizations of enzymes involved in human drug metabolism are urgently needed, which help to avoid severe adverse drug reactions. Advances are being made in understanding the role of drug-metabolizing enzymes in the control of PK, including individual isoforms of many enzymes such as cytochrome P450s (CYPs) and UGTs, and their selective substrates, inducers and inhibitors. Other non-P450 oxidative enzymes and conjugative enzymes are also discussed in this section since an increasing number of drugs are metabolized *via* these enzymes<sup>3</sup>.

#### 2.1.1. CYPs critical for PK

CYPs can oxidize foreign substances, enhance the water solubility and make drugs easier to be eliminated from the body. Most drugs are metabolized by CYPs, which mainly are located in the inner membrane of mitochondria or the endoplasmic reticulum of cells<sup>4</sup>. There are a total of 57 human CYP genes in 18 families. The members of CYP1 to CYP4 families oxidize thousands of exogenous and endogenous substrates (Table 1); whereas all members of CYP5 family and higher principally metabolize endogenous substrates in a highly substrate-specific manner<sup>5</sup>.

Most known chemical carcinogens, including aromatic amines and polycyclic aromatic hydrocarbons (PAHs), are substrates of CYP1 family, and their metabolism often results in the formation of active carcinogenic metabolites. In 2018, CYP1B1 was found in the mitochondria of cancer cells, where it reportedly metabolizes melatonin to form the metabolite *N*-acetylserotonin (NAS), which has antitumor effects<sup>6</sup>. CYP2D6, another important metabolic enzyme, is involved in the metabolism of many anti-cancer drugs, such as cyclophosphamide, tamoxifen, and gefitinib<sup>7</sup>. Recent research has found that in brain, CYP2D6 can metabolize both *m*-tyramine and *p*-tyramine into dopamine<sup>8</sup>. The CYP4 family has gained increasing attention for its potential to generate interesting metabolites and dispose of endogenous substrates in recent years. CYP4F11, together with CYP4F2, plays an important role in the synthesis of 20-hydroxyeicosatetraenoic acid (20-HETE) from arachidonic acid, and participates in the metabolism of vitamin K<sup>9</sup>. *Cyp2a5*, the mouse correlate of human *CYP2A6*, encodes an enzyme that exhibited circadian regulation<sup>10</sup>. The other CYP1 to CYP4 subfamilies are involved in metabolism of different endogenous and exogenous substrates, as listed in Table 1.

Understanding variation in mechanism-based enzyme activity is crucial for improving the clinical use of drugs. Highly selective inducers and inhibitors of CYPs have been cited in Guidance for Industry by FDA (<https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers>). Recent studies have revealed new chemicals and herb products as inducers or inhibitors of CYPs. For example, CYP7A1 is upregulated by an intestinal HIF-2 $\alpha$  inhibitor called PT2385<sup>17</sup>. The ketene intermediate of erlotinib can inactivate CYP3A4 and CYP3A5, which can result in liver injury<sup>18</sup>. Due to the complexity of components in the extract of herbs it is common that herb products exhibit different effects on the regulation of multiple enzymes. *Sophora flavescens* can inhibit CYP2B6, CYP2C8, CYP2C9, and CYP3A activities, while catalpol can inhibit the activity of CYP3A4, CYP2E1 and CYP2C9<sup>19,20</sup>. Other regulatory factors can also alter the expression of CYPs. For example, tumor suppressor p53 can regulate *Cyp2b10* directly and thereby attenuate APAP-induced hepatotoxicity<sup>21</sup>.

Herbs may be used singly or in combination in the treatment of diseases<sup>22</sup>. It is very important to understand how drug exposure alters molecular mechanisms underlying many complex drug interactions. For example, data show that ellagic acid from pomegranate peel guava leaf extract can significantly increase the AUC of warfarin with concomitant use. A significant reduction in CYP2C8, 2C9, and 3A4 activity was the main reason for this interaction<sup>23</sup>.

Based on recently available data, new information on the relative content of individual isoforms of P450 has been generated. Total CYP concentrations are significantly different between Chinese and Caucasian populations and the metabolic capabilities of CYPs in Chinese liver microsomes was significantly lower (<50%) in the  $CL_{int}$  for substrates of CYP1A2, CYP2C9, CYP2C19 and CYP2E1 than those of Caucasian populations<sup>24</sup>. Large variations in protein content, mRNA levels, and intrinsic activities of ten P450s (CYP3A4, 1A2, etc) have been revealed and some single nucleotide polymorphisms had significant impact on P450 expression; for example, CYP2C19 activity varied more than 600-fold<sup>25</sup>. A recent human PK study further evaluated CYP1A2 content in Chinese compared with Caucasian populations, enhancing the confidence in pharmacokinetic prediction of CYP1A2 content using two substrates (caffeine and theophylline)<sup>26</sup>.

Other organs like kidney and intestine also have significant metabolic capacity. There is definitive evidence for CYP2B6 and 3A5 expression in human kidney, while multiple CYPs are expressed in intestine<sup>27,28</sup>. The role of renal and intestinal enzymes in herbal product metabolism has been uncovered. Aminoglycoside antibiotics are leading causes for nephrotoxicity; combination with herbs or dietary supplements at reduced dosage is possible to reduce the risk of drug-mediated renal toxicity. A recent study revealed that moringa oleifera seed oil could limit gentamicin-induced oxidative nephrotoxicity<sup>29</sup>. Additional herbs have been identified as having effects on intestinal metabolism, such as the extracts of Yin-Chen-Hao Tang (YCHT), a very popular hepatoprotective three-herb formula in China and Japan<sup>30</sup>. These findings contribute to the understanding of the metabolic characteristics of renal and intestinal metabolism.

### 2.1.2. Non-P450 oxidative enzymes

The contribution of non-P450 enzymes to drug metabolism can be significant and affect the overall development of drugs. Non-CYP enzymes can be divided into four general categories: namely oxidative, reductive, conjugative, and hydrolytic. Non-CYP oxidative enzymes include flavin-containing monooxygenases (FMOs), monoamine oxidases (MAOs), peroxidases, xanthine oxidases (XO), aldehyde oxidase (AO), alcohol dehydrogenase (ADHs) and aldehyde dehydrogenase (ALDHs)<sup>31</sup>.

Very little is known about the regulation of content and activity of non-P450 oxidative enzymes. Recently, some selective substrates and inhibitors of non-P450 enzymes have been identified in natural products and other sources. FMOs are involved in the metabolism of a wide array of xenobiotics. Well-known inhibitors of FMOs include indole-3-carbinol and methimazole, and 2-mercaptobenzimidazole<sup>32</sup>. Classified into two different isoforms (MAO-A, MAO-B), MAOs are enzymes involved in the catabolism of monoamines. Benextramine and its derivatives were identified as novel human monoamine oxidase inhibitors, which could be considered as candidate drugs for the treatment of neurodegenerative diseases<sup>33</sup>. In addition, 3-(3-(dimethylamino)propanoyl)-7-hydroxy-5-methyl-2H-chromen-2-one hydrochloride has been found to function as a novel selective hMAO-B inhibitor, which is expected to be a promising multifunctional Parkinson's disease treatment agent<sup>34</sup>. XO and AO are involved in the oxidation of aldehydes and heterocycles, and carbazeran was used as a selective probe substrate of AO in hepatocytes<sup>35</sup>. Allopurinol and S-allyl cysteine (SAC) are XO inhibitors used in the treatment of gout and hyperuricemia<sup>36</sup>. A single-nucleotide polymorphism of human cytochrome P450 oxidoreductase (POR) in the Chinese population can regulate the content of POR and P450 isoforms<sup>37</sup>. Identifying specific inhibitor compounds will greatly facilitate investigation of enzyme-mediated drug disposition and drug interactions.

### 2.1.3. Importance of UDP-glucuronyltransferases (UGTs) in PK

UDP-glucuronyltransferases (UGTs) are a family of endoplasmic reticulum-bound enzymes which are responsible for the process of glucuronidation, a major part of phase II metabolism<sup>38</sup>. Human UGTs include 22 different functional enzymes and are classified into four gene families, UGT1, UGT2, UGT3 and UGT8<sup>39</sup>. The UGT1 and UGT2 families are primarily enzymes involved in drug glucuronidation, while the contribution of the UGT3 and UGT8 families to drug metabolism is minimal<sup>40</sup>.

Recently, UGT1A3 was found to be involved in the glucuronidation of alpinetin<sup>41</sup>. UGT1A4 is involved in the glucuronidation of metizolam<sup>42</sup>. Other UGT isoforms involved endogenous and exogenous substrates are listed in Table 2<sup>23,43–46</sup>.

**Table 1** Endogenous and exogenous substrates of CYPs and ligands of transcription factors.

Family	Enzyme	Endogenous substrate	Xenobiotic substrate	Transcription factor
CYP1	CYP1A1	Steroid (especially estrogen), aromatic amines, polycyclic aromatic hydrocarbons	Benzo[a]pyrene	AhR CAR
	CYP1A2	hydrocarbons	Phenacetin <sup>11</sup>	AhR, CAR
	CYP1B1	Steroid (especially estrogen), melatonin <sup>6</sup>	Aromatic amines, polycyclic aromatic hydrocarbons	AhR
CYP2	CYP2A6	Steroid	Nicotine, cotinine, coumarin <sup>12,13</sup>	PXR, NFE2L2, ER, GR, PXR, HNF4 $\alpha$
	CYP2A13	Unknown	Nicotine, coumarin, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) <sup>14</sup> , naphthalene <sup>15</sup>	FOXA2
	CYP2B6	Synthesis of cholesterol, steroids and other lipids.	Bupropion <sup>11</sup> , efavirenz	CAR, PXR, HNF4 $\alpha$
	CYP2C8	Arachidonic acid <sup>16</sup>	Paclitaxel, repaglinide, AZD9496, Taxol	CAR, PXR, ROR, VDR
	CYP2C9	Serotonin, polyunsaturated fatty acids, arachidonic acid.	Warfarin, phenytoin, tolbutamide	PXR, CAR, VDR, HNF4 $\alpha$
	CYP2C18	Arachidonic acid, linoleic acid, docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA).	Tolbutamide, cyclophosphamide, ifosfamide	Unknown
	CYP2C19	Arachidonic acid	S-Mephenytoin	PXR, CAR, FOXA3
	CYP2D6	Hydroxytryptamines, neurosteroids, <i>m</i> -tyramine, <i>p</i> -tyramine <sup>8</sup>	Tamoxifen, gefitinib, cyclophosphamide, bufuralol	HNF4 $\alpha$
	CYP2E1	Arachidonic acid	Chlorzoxazone (CHZ), acetaminophen	LXR, HNF1 $\alpha$ , NRF2
	CYP2F1	3-Methylindole (3MI)	Naphthalene, benzene, 1,1-dichloroethylene	Unknown
	CYP2J2	Arachidonic acid, vitamin D3	Astemizole	Unknown
	CYP2R1	Vitamin D3	Unknown	Unknown
	CYP2S1	Prostaglandin G(2)/H(2), thromboxane A(2), oxygenated eicosanoids	Benzo[a]pyrene-7,8-diol	Unknown
	CYP2U1	Arachidonic acid, docosahexaenoic acid (DHA)	Debrisoquin sulfate	Unknown
	CYP2W1	Fatty acids, lysophospholipids, retinoic acid	Canducarmycin	Unknown
CYP3	CYP3A4	Steroid (including testosterone), vitamin D3	Midazolam, rivaroxaban, 3-acetyl-11-keto- $\beta$ -boswellic acid (AKBA)	CAR, PXR, FXR, HNF4 $\alpha$ , LXR, VDR
	CYP3A5	Steroid (including testosterone), progesterone, Rostenedione	Diltiazem, cyclosporine, 3-acetyl-11-keto- $\beta$ -boswellic acid (AKBA)	PXR, LXR, HNF4 $\alpha$
	CYP3A7	Steroid (including testosterone)	3-acetyl-11-keto- $\beta$ -boswellic acid (AKBA)	Glucocorticoid receptor (GR), PXR
	CYP3A43	Androgen	Alprazolam	Unknown
CYP4	CYP4A11	Arachidonic acid, fatty acid, lauric acid	Unknown	PPAR $\alpha$
	CYP4A22			
	CYP4B1	Furan pro-toxin 4-ipomeanol	Pneumotoxin, 4-ipomeanol, aromatic amines, 2-aminofluorene	Unknown
	CYP4F2	Arachidonic acid, vitamin K menaquinone, leukotrienes, prostaglandins	Pafuramidine, fingolimod	Unknown
	CYP4F3	Arachidonic acid, prostaglandins, leukotriene-B4	Pafuramidine	Unknown
	CYP4F8	Arachidonic acid, prostaglandins, eicosanoids, dihomog- $\gamma$ -linolenic acid, leukotrienes, 19-hydroxylase of prostaglandin endoperoxides (PGEs)	Unknown	Unknown
	CYP4F11	Arachidonic acid, vitamin K menaquinone <sup>9</sup> , prostaglandins, leukotrienes	Benzphetamine, ethylmorphine, chlorpromazine, imipramine, erythromycin	RXR
	CYP4F12	Arachidonic acid, docosahexaenoic and eicosapentaenoic acids, prostaglandins, leukotrienes	Ebastine, terfenadine	PXR
	CYP4F22	Arachidonic acid, eicosanoids, prostaglandins, leukotrienes	Unknown	Unknown
	CYP4V2	Medium chain fatty acids	Unknown	PPAR $\gamma$
	CYP4X1	Arachidonic acid, anandamide	Unknown	PPAR $\alpha$
	CYP4Z1	Lauric acid, myristic acid	Unknown	Unknown



Highly selective substrates and selective inhibitors of UGTs have been found in natural products and from other sources. Resveratrol can activate UGT1A8 expression, and is used for breast cancer treatment<sup>47</sup>. Different doses of emodin can inhibit the activity of UGT2B7<sup>48</sup>.

In some cases, herbal products are metabolized by multiple UGTs. Linoleic acid and glutaric acid can inhibit the glucuronidation of berberrubine, a lipid-lowering metabolite of berberine, as well as the activities of UGT isoforms, such as UGT1A7, 1A8, 1A9<sup>49</sup>. Glucuronidation of catalposide, an active component of *Veronica* species, was catalyzed by gastro-intestine-specific UGTs 1A8 and 1A10<sup>50</sup>.

Gene polymorphisms are a key factor in the regulation of the content and activity of UGTs. UGT1A and UGT2B genetic variation can alter nicotine and nitrosamine glucuronidation in European and African American smokers<sup>51</sup>. In addition, the UGT1A4\*3 genetic polymorphism is associated with low posaconazole plasma concentrations in patients with hematological malignancies<sup>52</sup>. UGT1A1\*6 polymorphisms are correlated with irinotecan-induced neutropenia in cancer patients<sup>53</sup>.

#### 2.1.4. Other conjugative enzymes important for PK studies

In addition to UGTs, sulfonyl transferases (SULTs) and glutathione *S*-transferases (GSTs) are also important conjugative enzymes mediating phase II reaction.

SULTs catalyze the transfer of the water-soluble sulfonate group from 3'-phosphoadenosine 5'-phosphosulfate to drugs or endogenous molecules that contain hydroxy or amine group(s)<sup>54</sup>. At present, four families of human SULTs have been discovered, namely SULT1, SULT2, SULT4 and SULT6. SULT1E1 plays an important role in the metabolism and detoxification of estrogens and flavonoids<sup>55</sup>. SULT2 enzymes, mainly SULT2A and SULT2B, are primarily responsible for catalyzing the sulfation of hydroxysteroids<sup>56</sup>. A recent study found that tumor suppressor p53 could regulate the expression of SULTs<sup>57</sup>.

GSTs are a group of phase II drug-metabolizing enzymes that catalyze the binding of glutathione to various electrophilic compounds. In humans, cytosolic GST isoenzymes of the alpha, zeta, theta, mu, pi, sigma and omega classes have been found. GSTA1 plays a significant role in the metabolism of acetaminophen<sup>58</sup>. GSTA4 metabolizes electrophilic and carcinogenic substances such as endogenous carcinogen 4-hydroxy-2-nonenal<sup>59</sup>. The detailed substrates of SULTs and GSTs are listed in Table 3.

#### 2.1.5. Updates on the nuclear receptor-mediated regulation of xenobiotic-metabolizing enzymes

The human nuclear receptors comprise a family of 48 ligand-regulated transcription factors that in turn regulate target genes involved in metabolism and other physiological functions. Some of these receptors (*e.g.*, peroxisome proliferators-activated receptor (PPAR), liver X receptor (LXR), hepatocyte nuclear factor (HNF)) are of particular interest in regard to drug metabolism and disposition as they have been found to regulate many XMEs in recent years.

PPAR $\alpha$  induces the expression of CYP4A in response to a heterogeneous group of peroxisome proliferators. PPAR $\gamma$  also regulates the expression of CYP4V2, a fatty acid metabolizing enzyme, in human tetrahydropyranol 1 (THP1) macrophages<sup>60</sup>. LXR controls the transcription of *Cyp7a1* and *Cyp27a1*, *Cyp3a11* and *Cyp2e1*<sup>61–63</sup>.

Traditional transcriptional factors can bind directly to specific DNA sequences and thus control the gene expression. However, epigenetic regulation like histone modification and DNA

methylation modulates transcription of UGTs or CYPs mainly by changing chromatin architecture. For example, the *UGT1A* gene can be repressed by the recruitment of histones in females<sup>64</sup>. Several studies determined that microRNAs (miRNAs), could down-regulate the expression of metabolizing enzymes, which will be further reviewed in Section 2.3.

In summary, the expression and activity of metabolizing enzymes can be regulated by multiple factors, including drugs, nuclear receptors, gene polymorphisms, and even ethnic categories. Non-P450 enzymes and other conjugative metabolizing enzymes have gained attention in drug metabolism in recent years. It is desirable to illustrate the key factors responsible for variable expression and activity of drug metabolizing enzymes, as it may be beneficial in the prediction of potential therapeutics, drug–drug interactions, and in modifying the PK of drugs.

## 2.2. Transporters in the control of PK

### 2.2.1. Introduction of transporters

Transporters are membrane-bound proteins expressed on the cell membrane in most tissues with varying abundance. They can transport a variety of endogenous or exogenous substrates (such as drugs and their metabolites) in and out of cells. For drugs, transporters are the gatekeepers for cells and control the uptake and efflux of drugs. Transporters are involved in the ADME process of drugs. Therefore, transporters play critical roles in the pharmacokinetics, efficacy and toxicity of drugs. Alteration of transporter function or expression may significantly change the blood and/or tissue exposure of drugs, leading to significant changes in pharmacokinetics. Furthermore, the induction or inhibition of transporters by co-administered drugs can change PK and pharmacodynamics of therapeutic drugs and produce DDI.

There are more than 400 membrane transporters belonging to two major superfamilies: adenosine triphosphate (ATP)-binding cassette (ABC) and solute carrier (SLC) transporters. They utilize the energy that is released by ATP hydrolysis or an electrochemical ion gradient to translocate drugs across the membrane.

**2.2.1.1. The ABC family of drug transporters.** ABC transporters mainly act as exporters and pump drug molecules out of cells by utilizing the energy released by the hydrolysis of ATP. According to the organization and sequence of ATP-binding domains, 49 ABC transporters are classified into seven subfamilies: ABC1/ABCA, multidrug resistance (MDR)/TAP/ABCB, MRP/ABCC, ALD/ABCD, OABP/ABCE, GCN20/ABCF and White/ABCG<sup>65</sup>. Among them, P-glycoprotein (P-gp, MDR1, ABCB1), MRPs/ABCCs, breast cancer resistance protein (BCRP/ABCG2) and bile salt export pump (BSEP/ABCB11) are recognized for their importance in drug disposition<sup>66</sup>. P-gp, which is expressed at a high level in the intestine, liver, kidney, brain and placenta, is the most studied ABC transporter. Many substrates of P-gp including antibiotics, statins, immunosuppressants, anticancer drugs and a broad spectrum of drugs overlap with the substrates of CYPs. The expression of P-gp is regulated by several transcription factors including PXR, CAR, vitamin D receptor (VDR) and CCAAT/enhancer binding protein (C/EBP) and some microRNA such as miR-451, miR-27a and miR-145<sup>67,68</sup>. Furthermore, P-gp is usually overexpressed in cancer cells and plays a critical role in MDR. For example, during chemotherapy, P-gp may be an obstacle for drug exposure if the therapeutic drugs are P-gp substrates<sup>69</sup>. Besides its

**Table 2** Endogenous and exogenous substrates of UGTs and ligands of transcription factors.

Family	Enzyme	Endogenous substrate	Xenobiotic substrate	Transcription factor
UGT1A	UGT1A1	Bilirubin, estradiol, fatty acids	SN-38, leonurine, bergenin, axitinib	CAR, PXR, PPAR $\alpha$ , AhR <sup>43</sup> , NRF2
	UGT1A3	Bile acid, arachidonic	Polyaromatic amines, non-steroidal anti-inflammatory drugs, statins, ahydroxygenkwanin, genkwanin, ursolic acid <sup>44</sup> , fimasartan <sup>45</sup> , alpinetin <sup>23</sup>	PPAR $\alpha$ , HNF1, AhR, LXR, PXR
	UGT1A4	Eicosanoids	Imipramine, lamotrigine, clonazepam, deschloroetizolam, etizolam, flubromazolammetizolam	HNF1, PPAR $\alpha$ , PXR, CAR, AhR, HNF1 $\alpha$
	UGT1A6	Serotonin	1-Naphthol 4-nitrophenol	AhR, CAR, PXR, PPAR $\alpha$
	UGT1A7	Unknown	Icaritin, carcinogens	AhR, HNF1, HNF4 $\alpha$ , NRF2
	UGT1A8	Fatty acids	Retinoids, catechol estrogens, opioids, coumarins, flavonoids, anthraquinones, phenols, raloxifene	HNF1, HNF4 $\alpha$ , AhR, NRF2
	UGT1A9	Steroids, fatty acids	Bulky phenols, propofol, mycophenolic acid, niflumic acid, psoralidin	CAR, HNF1, HNF4 $\alpha$ , PPAR $\alpha$ , AhR, NRF2
	UGT1A10	Estrogens	Nitrosamine, flavonoids, polycyclic aromatic hydrocarbons, raloxifene, dopamine	HNF1 $\alpha$ , HNF4 $\alpha$ , AhR, NRF2
UGT2A	UGT2A2/3	Hyodeoxycholic acid	Tobacco carcinogen	HNF1, LXR
UGT2B	UGT2B4	Arachidonic acid	Naftopidil, deoxynivalenol	PPAR $\alpha$ , AhR, FXR
	UGT2B7	Sex-steroid hormones, glucocorticoids, mineralocorticoid, bile acids	Naftopidil, deoxynivalenol, mirabegron, efavirenz, zidovudine, codeine, morphine	HNF1 $\alpha$ <sup>46</sup> , CAR, PXR, FXR, PPAR, NRF2
	UGT2B10	Eicosanoids	Amitriptyline, imipramine, clomipramine, trimipramine	CAR, FXR, AR
	UGT2B11	Unknown	3 $\alpha$ -Hydroxyandrogens, 3 $\alpha$ -pregnanes, Hydroxylestrogens	ER, AR
	UGT2B15	Sex-steroid hormones	Oxazepam, lorazepam, sipoglitazar, bisphenol-A	AR, ER, HNF3 $\alpha$ , FXR
	UGT2B17	Sex-steroid hormones	Coumarins, anthraquinones flavonoids, chlorantraniliprole	HNF1 $\alpha$ , HNF4 $\alpha$ , HNF3 $\alpha$ , AR, ER
	UGT2B28	Sex-steroid hormones	Unknown	ER, AR
UGT3	UGT3	Unknown	N-Acetylglucosamine	Unknown
UGT8	UGT8A1	Bile acids	Unknown	LXR

role in MDR induction, P-gp plays a critical role in pharmacokinetics, pharmacology and toxicology. Through pumping multiple drugs out of cells, P-gp decreases the bioavailability of oral drugs and increases drug efflux into urine or bile. Furthermore, P-gp also plays a vital role in the maintenance of the blood–brain barrier by pumping drugs or toxins out of the CNS<sup>70</sup>. Another important ABC transporter group is the MRP family that consists of 9 MRP proteins (MRP1–MRP9). Among them, MRP2 is important in drug pharmacokinetics. MRP2, once known as the canalicular multispecific organic anion transporter, is highly expressed in liver, intestine and kidney. Chemotherapeutics such as methotrexate, melphalan, and statins are the classical substrates of MRP2. Since co-expressed in the liver, many liver-enriched transcription factors such as LXR, farnesoid X receptor (FXR), HNF and C/EBP regulate the transcription of MRP2. Another efflux transporter BCRP is a half transporter and is expressed at a high level in a wide variety of tissues such as intestine, kidney, liver, testis and brain. BCRP is modulated by the progesterone receptor B (PRB) and estrogen receptor (ER). Another ABC family drug transporter, BSEP, is primarily expressed in the liver and pumps bile acids and non-bile acid drugs such as pravastatin into bile.

**2.2.1.2. The SLC family of drug transporters.** The SLC family consists of 55 gene subfamilies and more than 360 family

members. SLC transporters mainly utilize the energy stored in the ion gradients across membranes, but do not depend directly on ATP hydrolysis<sup>71</sup>. Several SLC family transporters play important roles in drug disposition including organic anion-transporting proteins (OATPs/SLC21/SLCO), organic anion and cation transporters (OATs and OCTs/SLC22), peptide transporters (PEPTs/SLC15) and sodium-dependent bile acid transporters (NTCP/SLC10A1). The OATP family consists of 11 members. Among them, four transporters including OATP1A2 (SLCO1A2), OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3) and OATP2B1 (SLCO2B1) are involved in drug transport<sup>72</sup>. OATP1A2 is expressed in the intestinal epithelium, renal epithelium and brain capillary endothelial cells, while OATP1B1, OATP1B3 and OATP2B1 are expressed predominantly in hepatocytes. Statins and anti-cancer drugs like paclitaxel, sorafenib and methotrexate are known as the substrates of OATPs. The SLC22 family consists of 23 members, including OCTs, zwitterion/cation transporters (OCTNs) and OATs. Among OCTs, OCT1 (SLC22A1) is mainly expressed in the liver, OCT2 (SLC22A2) is located at a high level in proximal tubular cells, and OCT3 (SLC22A3) has a broader expression range. Several drugs have been identified as OCT substrates including anesthetic drugs, the anti-diabetic drug metformin, antidepressants,  $\beta$ -blockers and anti-cancer chemotherapeutics. Among OATs, OAT1 (SLC22A6) and OAT3 (SLC22A8) have a broader expression range with the highest expression in

**Table 3** Endogenous and xenobiotic substrates for GSTs and SULTs that are also ligands of particular transcription factors.

Enzyme	Endogenous substrate	Xenobiotic substrate	Transcription factor
GSTs	Steroids, bilirubin, heme, fatty acids	1-Chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), 4-nitrobenzyl chloride (pNBC), ethacrynic acid (ETHA), aflatoxin B1 (AFB1), 4-hydroxynonenal (4HNE), acrolein, <i>N</i> -acetyl- <i>p</i> -benzoquinone imine (NAPQI), cisplatin, busulfan, dichloroacetate, cyclophosphamide, azathioprine (AZA)	PXR, CAR, steroidogenic factor 1 (SF-1), RXR
SULT1	SULT1A1 4-Methylphenol, iodothyronines	4-Nitrophenol	PXR, CAR
	SULT1A2 Dopamine, estrogens, catechol estrogens	4-Nitrophenol, 2-naphthol, naloxone, minoxidil	PXR, CAR, FXR, HNF4 $\alpha$
	SULT1A3 Dopamine, norepinephrine, iodothyronines	6-Hydroxydopamine, hydromorphone	
	SULT1B1 Thyroxine	3-OHB[a]P, 1-naphthol	
	SULT1C1 Thyroxine	<i>N</i> -Hydroxyarylamines	
	SULT1E1 Iodine thyroxine, pregnenolone	1-Naphthol, naringenin, genistein, 4-hydroxytamoxifen	
SULT2	SULT2A Dehydroepiandrosterone (DHEA), bile acid, cholesterol, estrone	Tibolone, budesonide	
	SULT2B Dehydroepiandrosterone (DHEA), bile acid, cholesterol, estrone	3 $\beta$ -Hydroxysteroids	

kidney, while OAT2 (SLC22A7) is primarily expressed in the liver. OAT1 substrates include antiviral drugs, antibiotics, diuretics and angiotensin-converting enzyme (ACE) inhibitors. For the SLC15 subfamily, PEPT1 and PEPT2 are the most studied transporters. Both mediate oligopeptide uptake. PEPT1 is highly expressed in the intestine and mediates drug absorption, while PEPT2 is mainly expressed in kidney and affects renal reabsorption.

### 2.2.2. Transporters are critical for PK

The ADME process determines the blood and tissue concentration of drugs, as well as subsequent pharmacological or toxicological effects. The intestine and liver, both of which tightly regulate the entry of drugs into the blood circulation, are important organs in determining the bioavailability of oral drugs. Elimination of drugs or their active metabolites occurs either by metabolism to inactive metabolites that are excreted, or by direct excretion of drugs or active metabolites in the kidney. The transporters expressed in intestine, liver and kidney are involved in the absorption, distribution and excretion processes of drugs, and are the major determinant in blood and tissue concentration of drugs.

#### 2.2.2.1. Transporter-mediated oral drug absorption.

Oral drug absorption primarily occurs in the intestine, which is the major determinant of drug bioavailability, together with the first-pass extraction in the liver. Drug molecules pass through the membranes in the intestine through two pathways: passive diffusion and transporter-mediated absorption.

The process of transporter-mediated oral drug absorption consists of two parallel transport processes including transporter-mediated uptake and transporter-mediated efflux<sup>73,74</sup> (Fig. 1A). In general, net drug absorption depends on multiple uptake and efflux transporters in the intestine. Uptake transporters such as OATP2B1, PEPT1 and sodium-dependent bile acid transporter (ASBT/SLC10A2) are involved in the intestinal uptake of drugs across the brush border membrane<sup>75</sup>. For example, PEPT1 transports di/tripeptides-like anticancer drugs such as bestatin and  $\beta$ -

lactam antibiotics into enterocytes<sup>76–78</sup>. Efflux transporters expressed on the brush border membrane of the intestine, are considered as the barriers for intestinal drug absorption. P-gp, MRP2 and BCRP are three major efflux transporters in the intestine. P-gp, the most studied efflux transporter, has broad substrate specificity and significantly limits the bioavailability of many oral drugs<sup>79</sup>. For example, co-treatment with verapamil, a P-gp inhibitor, increases the intestinal absorption of afatinib or bestatin due to P-gp inhibition in the intestine<sup>80,81</sup>. On the contrary, rifampin, a P-gp inducer, decreases the oral absorption of cyclosporine and tacrolimus through the induction of P-gp in the intestine<sup>82</sup>. BCRP is another efflux transporter expressed in the intestine and suppresses the intestinal absorption of drugs<sup>83</sup>. Due to only one ATP binding site and six putative transmembrane helices, BCRP is considered a “half-transporter”. The substrates of BCRP include statins (pitavastatin, rosuvastatin), antiviral drugs (lamivudine, zidovudine, abacavir), anticancer drugs (methotrexate, SN-38, irinotecan, gefitinib, imatinib, erlotinib) and antibiotics (nitrofurantoin, ciprofloxacin)<sup>84</sup>. The efflux transporter MRP2 is also expressed on the brush border membrane of the intestine and transports a variety of substrates conjugated with sulfate, glutathione and glucuronide, as well as various unmodified drugs. Previous studies showed that resveratrol inhibited MRP2 and thereby increased the intestinal absorption of methotrexate<sup>85</sup>.

#### 2.2.2.2. Transporter-mediated drug distribution.

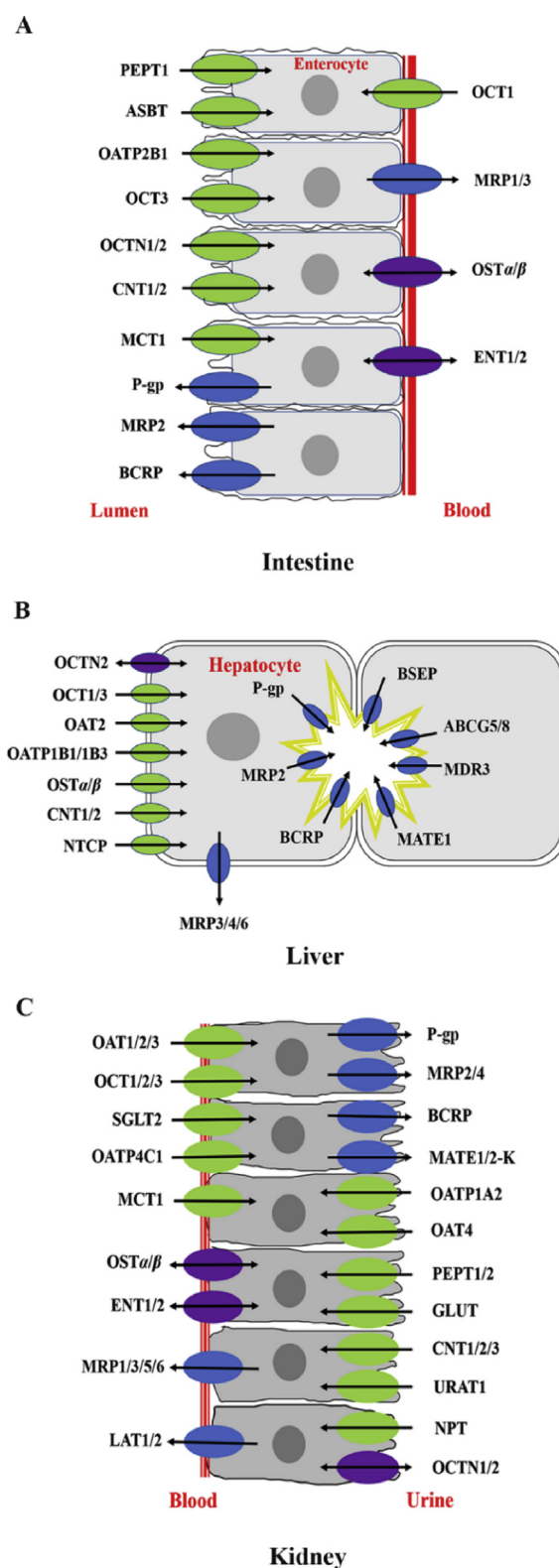
Transporters also affect the tissue distribution and contribute to the selective distribution of drugs to specific tissues. For example, OATP1B1 and OATP1B3 are the major uptake transporters in the liver for cilostazol, and MRP2, BCRP, P-gp pump cilostazol out of the liver into bile<sup>86</sup>. These transporters assist the liver-specific distribution of cilostazol. Another example is pravastatin, which enters into the liver through OATP1B1 and OATP1B3. After being excreted into the bile, pravastatin is reabsorbed in the intestine to the portal vein and taken up by the liver, and effectively undergoes enterohepatic circulation<sup>87</sup>. Therefore, the liver concentration should be higher



than that in the circulating blood, leading to a high pharmacological effect at a relatively low plasma concentration. Transporters are also expressed on the blood–brain barrier and play critical roles in restricting the distribution of drugs into the brain. Increasing evidence has demonstrated that P-gp on the blood–brain barrier can suppress the distribution of drugs into the CNS<sup>88,89</sup>. Also, BCRP is recognized as an efflux transporter on the blood–brain barrier suppressing drug entry into the brain. Except for efflux transporters, uptake transporters are also expressed on the blood–brain barrier and play key roles in the uptake of neuroactive drugs. OAT3 is highly expressed on the basolateral membrane of brain capillaries<sup>90</sup>, and OCT2 is expressed in neurons and the choroid plexus. OCT2 is involved in the reabsorption of many drugs such as serotonin, norepinephrine, dopamine, choline and histamine from the cerebrospinal fluid.

**2.2.2.3. Transporter-mediated drug excretion.** Drug elimination primarily occurs in the liver and kidney. Hepatobiliary elimination processes can be summarized as follows: (1) the uptake of drugs into hepatocytes *via* uptake transporters or passive diffusion; (2) drug metabolism in hepatocytes including CYP metabolism (phase I metabolism) and conjugation (phase II metabolism); (3) excretion from hepatocytes into bile or portal blood *via* efflux transporters. Hepatobiliary transport of drugs is attributable to transporters located on the basolateral (sinusoidal) or canalicular (apical) membrane of hepatocytes (Fig. 1B). SLC superfamily transporters are responsible for drug uptake from the portal blood into hepatocytes. Among them, OAT2, OCT1, OATPs and NTCIP are major uptake transporters. Efflux transporters such as P-gp, BCRP, MRP2 and BSEP are responsible for the hepatobiliary excretion of drugs and their metabolites. In addition, the efflux transporter MRP3, 4 and 6 expressed on the basolateral membrane are responsible for the basolateral efflux of drugs from the liver into the blood circulation. The hepatic transporters OAT2, OATP1B1/1B3 and OCT1 are highly expressed in the liver and are considered to be of particular importance for hepatic drug elimination, PK and efficacy. Much like the interplay of transport and metabolic enzymes at the intestinal barrier, these transporters also have a “gatekeeper” function in the drug movement from the blood into hepatocytes; they regulate both the number of drugs available for metabolism by liver enzymes and the subsequent biliary excretion. Efflux transporters including P-gp, BCRP, MRP2 and BSEP are responsible for the biliary excretion of endogenous and exogenous molecules. Many studies have shown that P-gp transports amphiphilic cationic drugs such as doxorubicin, digoxin and vinblastine into bile<sup>91</sup>. BCRP is involved in the biliary excretion of sulfated conjugates of steroids and drugs such as doxorubicin, mitoxantrone and daunorubicin, while BSEP transports drugs including vinblastine and taxol, et al. Due to their important roles in hepatobiliary efflux, the inhibition of BSEP, BCRP and MRP2 may lead to cholestasis. Therefore, the effects of chemicals on transporter-mediated hepatobiliary excretion must be determined in drug discovery<sup>92</sup>.

The kidney is the major organ of drug excretion. Renal clearance of drugs consists of glomerular filtration, tubular secretion and reabsorption. The proximal tubule region is responsible for the active secretion and reabsorption of drugs. Many transporters are located at the renal tubular epithelial cells and are involved in the proximal tubular secretion and reabsorption (Fig. 1C). These transporters include OCTs, OATs, multidrug



**Figure 1** Drug transporter expression in tissues. Drug transporter expression in the intestine (A), liver (B) and kidney (C). The arrows indicate the general directions in which the substrates are transported.

and toxin extrusion proteins (MATE1 and MATE2-K), sodium-phosphate transporter (NPT/SLC17A1), OATPs and PEPTs, as well as equilibrium and concentration nucleoside transporters (ENTs and CNTs/SLC28A). Among them, OCTs, OATs and MATEs play critical roles in the active secretion of renal proximal tubule. These transporters work in concert with efflux transporters to transfer drugs into urine. OATs mainly transport anionic drugs such as beta-lactam antibiotics and anti-inflammatory drugs. The competitive inhibition of OATs may lead to a decrease in renal tubular secretion and an increase in the systemic concentration of drugs. For example, co-administration of probenecid, one OAT inhibitor, decreases renal secretion, leading to an increase in the plasma concentration of bestatin<sup>93</sup>. JBP485, a dipeptide with potential protective activity against kidney, liver and intestinal injury, has been demonstrated to be a substrate of OATs. Co-administration of JBP485 and cephalexin decreased the accumulative renal excretion and renal clearance of both compounds<sup>77</sup>. When JBP485 and lisinopril were co-administered, the competitive inhibition of OAT1 and OAT3 were also observed in OAT1/3-HEK293 cells<sup>94</sup>. In addition, acyclovir, an antiviral drug, was also a substrate of OAT1/3 and JBP485 can inhibit its renal excretion<sup>95</sup>. Furthermore, the DDIs between JBP485 and entecavir through the competitive inhibition of OAT1 and OAT3 significantly decreased the renal excretion of both compounds<sup>96</sup>. On the other hand, OATs are involved in drug-related nephrotoxicity. Probenecid, by inhibiting OAT1 and OAT3, reduced the accumulation of cephaloridine and subsequently nephrotoxicity<sup>97,98</sup>.

Three OCT isoforms including OCT1, OCT2 and OCT3 have been found in the kidney. Among the three OCTs, OCT2 is the major transporter for renal secretion of a variety of drugs such as memantine, metformin and amantadine. DDIs may also occur through the competitive inhibition of OCTs. For example, through inhibiting OCT2, cimetidine decreases the renal excretion of metformin and increases its plasma concentration<sup>99</sup>. On the other hand, OCT2, by modulating the exposure of drugs to renal proximal tubule cells regulates the nephrotoxicity of anticancer drug cisplatin and its analogs<sup>100</sup>. Substrates taken up from the systemic circulation may subsequently undergo efflux across the brush border membrane of proximal tubule cells by various ABC efflux transporters such as P-gp and BCRP. For example, a probe P-gp substrate, methotrexate, is actively secreted into urine. Co-treatment with bestatin, another P-gp substrate, increases plasma concentrations and decreases the renal clearance of methotrexate<sup>101</sup>. MATE1 and MATE2-K are expressed on the brush border membrane of proximal tubular cells. MATE1 mediated the renal secretion of fluoroquinolones including gatifloxacin, ciprofloxacin, levofloxacin, enoxacin, pazufloxacin, norfloxacin and tosufloxacin.

In summary, the expression and activity of transporters can be regulated by drugs and competitive inhibition may occur after co-administration of more than one drug. Furthermore, species differences in transporters complicate pharmacokinetic scaling from preclinical species to humans. Additionally, the expression of transporters may also be regulated by disease progression<sup>102</sup>. Modulation of transporter expression by disease states can potentially modify the PK of drugs.

### 2.3. ncRNAs in the regulation of drug metabolism and pharmacokinetics

ncRNAs are genome-derived RNA molecules that are not translated into proteins. Indeed, the human genome is comprised of over 95%

of noncoding sequences<sup>103</sup> that are transcribed into various forms of functional ncRNAs including miRNAs, transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nucleolar RNAs (snoRNAs), and long noncoding RNAs (lncRNAs). Among them, miRNAs usually lead to translation inhibition or enhance mRNA degradation in cells through complementary base pairing with target transcripts. Many miRNAs have been shown to modulate the expression of drug-metabolizing enzymes or transporters, and consequently alter cellular drug metabolism and transport capacity, as well as drug responses (see recent reviews<sup>104–106</sup>). For instance, miR-27b reduces CYP1B1 protein expression in human carcinoma cells and thus suppresses CYP1B1 enzymatic activity, as indicated by a P450-Glo™ luminescent assay<sup>107</sup>. Meanwhile, miR-27b modulates CYP3A4 expression through direct targeting of CYP3A4 3'-untranslated region (3'UTR) and "indirect" targeting of transcriptional factors such as NR1I1/VDR<sup>108</sup>, which may significantly alter CYP3A4-mediated drug metabolism<sup>109,110</sup>. Furthermore, miR-27a/b regulates the expression of a number of transporters such as ABCB1/P-gp<sup>111–113</sup>, and thus influences intracellular drug accumulation and chemosensitivity. In addition, a number of Phase 2 drug-metabolizing enzymes such as the UGTs are regulated by miRNAs at the posttranscriptional level<sup>114–119</sup>. Findings on miRNA-controlled regulation of DMPK provide new insights into mechanisms behind inter-individual variations in pharmacotherapy.

Recent studies on miRNA regulation in DMPK also led to the development of novel research approaches and technologies. For example, while the luciferase reporter assay, gene mutagenesis and correlation analysis are helpful methods for the assessment of the interactions between miRNAs and target transcripts, a more direct approach has been established which is based on the change of RNA mobility after binding to miRNA, namely RNA electrophoretic mobility shift assay (EMSA)<sup>120,121</sup>. Using this RNA EMSA and other methods, a number of CYP genes (*e.g.*, *CYP2C19*, *CYP2E1* and *CYP2D6*) and regulators have been shown to be regulated post-transcriptionally by particular miRNAs<sup>121–125</sup>. It is also noteworthy that miRNA research is limited to the use of miRNA-expressing plasmids or viruses, or chemically-synthesized or chemo-engineered miRNA mimics<sup>126–128</sup>. To better capture the properties of biologic RNA molecules and cellular miRNA machinery, a novel RNA bioengineering technology has been established for the production of biologic miRNA agents in living cells<sup>109,129–133</sup>. With such novel bioengineered miRNA agents produced cost effectively and on a large scale, extensive functional studies have been conducted and the results showed rather a modest change in the PK of major CYP probe drugs in mouse models<sup>134</sup>. Further studies have demonstrated the utility of miRNAs as therapeutics or sensitizing agents for the treatment of human diseases in various animal models<sup>133,135–139</sup>.

There is also growing evidence that lncRNAs may regulate the expression of drug-metabolizing enzymes and transporters. For example, expression of HNF1 $\alpha$  antisense RNA 1 (HNF1 $\alpha$ -AS1) and HNF4 $\alpha$  antisense RNA 1 (HNF4 $\alpha$ -AS1) shows a significant influence on the basal and drug-induced expression of drug-metabolizing enzymes in human cells<sup>140</sup>. H19, an lncRNA highly expressed in liver tissues, induces the expression of efflux transporter P-gp/MDR1/ABCB1 in drug-resistant HepG2 cells<sup>141</sup>. The lncRNA MRUL confers the overexpression of ABCB1 in drug-resistant gastric cancer cells<sup>142</sup>. Furthermore, some studies have demonstrated that a lncRNA modulates drug sensitivity through its action on miRNA-transporter axis<sup>143,144</sup>. In addition, as RNA editing and posttranscriptional modifications are critical

for RNA stability and biological function, very recent studies have also demonstrated the alteration of DMPK gene expression following RNA editing<sup>145–147</sup>. Future studies in these areas will undoubtedly advance our understanding of RNA-based regulation in DMPK.

In summary, research on miRNA-controlled regulation of DMPK provides new insights into understanding the post-transcriptional regulatory mechanisms behind inter-individual variations. Novel technologies and research approaches are also established during the investigation of ncRNA regulation of DMPK gene expression, which should have broad impact on biomedical research. Evidence is accumulating that some lncRNAs may be involved in the regulation of DMPK, which represents a new area of research.

### 3. Drug–drug interactions

#### 3.1. Current status of research on drug–drug interactions

DDIs may result in favorable or toxic effects. Patients frequently use more than one medication at a time. Depending on the clinical settings and the number of drugs prescribed, the incidence of potential DDIs ranges between 15% and 80%<sup>148</sup>. DDIs can be classified mechanistically into 3 major types: physio-chemical incompatibility, PK interactions, and pharmacodynamic interactions<sup>149</sup>. Physio-chemical interactions usually occur when positively and negatively charged compounds are mixed before they are administered or absorbed. Pharmacokinetics-based DDIs, characterized by altered concentration of unbound drugs that exert pharmacological effects, can be caused by several mechanisms, including: 1) alteration of drug metabolizing enzymes (*e.g.*, CYPs)<sup>150</sup>, 2) alteration of transporters involved in the absorption, distribution and excretion of drugs (*e.g.*, MDR1, OAT, OCT, etc)<sup>150</sup>, 3) influence on plasma protein binding affinity<sup>149</sup>, and 4) changes in the function of organs (*e.g.*, gut motility or stomach content pH)<sup>149</sup>. Pharmacodynamics-based DDIs are characterized by a shift of the unbound drug concentration *versus* response curve<sup>149</sup>. New responses that are not present when either of the drugs is given alone may also be observed when drugs are used in combination.

*In vitro*, *in vivo* and clinical studies are usually conducted to identify any potential DDIs. The *in vitro* studies are usually simple systems that can be used for high throughput screening and provide mechanistic information for potential DDIs. *In vivo* animal studies are often conducted using clinically relevant dosages and pharmacodynamic endpoints to confirm the *in vitro* observations. If evidence obtained from *in vitro* and *in vivo* animal models suggests strong DDIs potential further clinical trials are recommended<sup>150,151</sup>. Recently, mathematical modeling, particularly physiologically-based pharmacokinetic (PBPK) modeling has also been applied to investigate potential pharmacokinetic-based DDIs. A recent review by Min et al.<sup>152</sup> depicted how pharmacokinetic modeling improves and simplifies the investigation on DDIs. In addition, systematic reviews and databases summarize all the experimental and predicted data on DDIs, which are useful for providing warning and proper advice to patients in clinical practice<sup>153</sup>.

Although DDIs between small molecule drugs have been well investigated and documented, knowledges on interactions between drugs and herbs, interactions between therapeutic biologics, and

interactions mediated by the gut microbiome are currently not well understood. The cutting-edge investigations on these aspects are briefly introduced in the following sections.

#### 3.2. Current status of research on herb–drug interactions

Herbal plants and herbal products are commonly used as remedies and dietary supplements. When herbs are concurrently administered with drugs unrecognized herb–drug interactions (HDIs) may lead to side effects and toxicity. HDIs basically share the same mechanisms as DDIs. To avoid physio-chemical interactions between herbal components and drugs, it is usually recommended that herbs should be taken at two hours before or after the drugs. Moreover, herbs may sometimes alter the PK and/or pharmacodynamics of the concurrently administered drugs. PK and pharmacodynamic interactions have been reported between herbs and drugs with narrow therapeutic indexes, especially drugs for CNS and cardiovascular diseases<sup>154</sup>. For example, St John's wort (*Hypericum perforatum*) was reported to decrease warfarin plasma concentrations *via* inducing the activity of CYPs, leading to the loss of anticoagulant activity<sup>155</sup>. A traditional Chinese herb Danshen (*Salvia miltiorrhiza*) was reported to interact with warfarin on both its PK profiles and pharmacodynamic effects, resulting in over-anticoagulation and increased risk of bleeding<sup>155</sup>.

Investigation of HDIs is often more complicated than those of DDIs, due to the complex herbal components and the batch-to-batch variation of herbal products. As demonstrated in Table 4, compared with DDIs, research on HDIs is still insufficient. *In vitro* screening assays, which are efficient ways for detecting potential DDIs, may not be applicable for testing crude herbs or herb extracts, due to the fact that some of the herbal components may not be bioavailable, and adding such herbal components to the *in vitro* cell/microsome systems may alter results. By using LC–MS/MS, several multi-compound pharmacokinetic studies allowed the simultaneous detection of the plasma/tissue concentrations of multiple components after ingestion of the studied herb, facilitating the discovery of the bioavailable active components and subsequent *in vitro* and *in vivo* mechanistic studies on potential HDIs<sup>156,157</sup>. Most of the reported HDIs are based on *in vitro* and *in vivo* animal models, providing evidence with low clinical relevance. Moreover, many clinical studies were conducted among healthy populations, where the impact of the herbs on the pharmacodynamics effects of the concurrent drug may not be determined. On the other hand, the wide variation between different batches of herbal products also leads to poor reproducibility of the tests. Although not true in all countries, herbal products in China are generally regulated and used as medicine with standardization of the content of the major active components, and the herbal products are sometimes investigated not only as the effector but also as the affected agent of HDIs. In addition to experimental approaches based on the pre-clinical and clinical data, mathematical models have been established to predict HDIs, demonstrating the feasibility of using PBPK modeling for the prediction of HDIs<sup>152</sup>. For example, PBPK modeling of two major active components from Wuzhi capsule (*Schisandra sphenanthera* extract) predicts its interaction with tacrolimus metabolism by CYP3A4 inhibition<sup>158</sup>. However, the application of modeling and simulation on the investigation of HDIs is still restricted by the limited human pharmacokinetic data of herbal components<sup>152</sup>. More sophisticated designs of clinical studies are warranted to evaluate the safety and efficacy of the concomitant use of herbs and drugs.

### 3.3. Trends in drug–drug interactions of therapeutic biologics

Therapeutic biologics include therapeutic proteins, monoclonal antibodies (mAbs), vaccines, and peptide and nucleic acid derivatives that are manufactured for pharmaceutical uses<sup>159</sup>. Development of therapeutic biologics is growing fast, and in clinical practice the risk of DDIs with biologics is increasing.

#### 3.3.1. PK of therapeutic biologics

The PK of biologics is different from those of small molecules. Since most therapeutic biologics undergo rapid degradation in the gastrointestinal tract after oral administration, alternative routes, such as intravenous, intramuscular, and subcutaneous injection are often used for drug delivery<sup>159–161</sup>. The distribution of therapeutic biologics is mainly mediated by interstitial penetration, lymphatic drainage, transcytosis, and receptor-mediated cell uptake<sup>159–161</sup>. Therapeutic proteins usually have a limited volume of distribution and do not bind to plasma proteins, and their biliary and renal excretion is generally negligible<sup>162</sup>. Catabolism *via* proteolytic degradation is the predominant clearance pathway for most therapeutic proteins<sup>159–161</sup>, while target antigen-mediated disposition also plays a role<sup>161</sup>. Moreover, fragment crystallizable receptor (FcR)-mediated antibody recycling by monocytes, macrophages, and dendritic cells is a salvage pathway that prolongs the half-lives of many mAbs<sup>159–161</sup>. Immune responses participate in both the catabolism and the antibody recycling process, and therefore immunogenicity can significantly influence the clearance of therapeutic proteins<sup>159</sup>. A recent review by Ferri et al.<sup>162</sup> has summarized the

pharmacokinetic DDIs of therapeutic antibodies. Unlike therapeutic proteins, nucleic acid and peptide drugs<sup>160</sup> are rapidly eliminated by peptidases and nucleases<sup>159,163</sup>, and may also undergo slow renal excretion<sup>161</sup>. Plasma binding of these oligomers can sometimes be very high and has been reported to affect their distribution and clearance<sup>160</sup>.

#### 3.3.2. Pharmacokinetics-based interactions of therapeutic biologics

Direct competition between therapeutic biologics and small molecules in PK is not common due to their distinct pharmacokinetic pathways<sup>163</sup>. However, certain indirect pharmacokinetic DDI may occur. Immunosuppressive agents may decrease the immunogenicity of the therapeutic protein so as to hinder its clearance<sup>163</sup>. For example, concomitant treatment with the immunosuppressant methotrexate can decrease the clearance of mAbs including golimumab<sup>164</sup>, adalimumab<sup>162</sup>, and infliximab<sup>165</sup>. Another indirect pharmacokinetic DDI mechanism is cytokine–CYP modulation. Several biologics with immunomodulatory effects may alter CYP activities *via* modulating the cytokine levels leading to the altered PK of co-administered small molecules that are substrates of the affected CYPs<sup>159,163,166</sup>. For instance, tocilizumab, which can induce CYP3A4 activity by decreasing interleukin 6 levels, was found to reduce simvastatin systemic exposure<sup>167</sup>. Similarly, by triggering inflammation, influenza vaccination has been reported to decrease CYP activity and thus influence the systemic exposure of CYP substrates such as clozapine<sup>168</sup>. PBPK modeling is a powerful tool for the investigation of pharmacokinetic-based interactions between therapeutic biologics

**Table 4** Comparison between investigations on DDIs and HDIs<sup>151,153</sup>.

Type of investigation	DDIs	HDIs
<i>In vitro</i> studies	<ul style="list-style-type: none"> <li>Commonly used for the screening of potential DDIs.</li> <li>Provide mechanistic information.</li> </ul>	<ul style="list-style-type: none"> <li>Single component/artificial mixture of major components used in a test.</li> <li>Does not account for bioavailability.</li> <li>Provide mechanistic information for certain components.</li> </ul>
<i>In vivo</i> animal studies	<ul style="list-style-type: none"> <li>Drugs tested in clinically relevant doses.</li> <li>Provide pharmacokinetics and pharmacodynamics information for clinical trials.</li> </ul>	<ul style="list-style-type: none"> <li>Crude herbs or herb extracts tested in clinically relevant doses.</li> <li>Address bioavailability of the herbal components.</li> </ul>
Clinical studies	<ul style="list-style-type: none"> <li>Retrospective evaluation may not provide sufficient precision to assess DDIs.</li> <li>Clinical trials on healthy volunteers for pharmacokinetics-based DDIs.</li> <li>Pharmacodynamics-based DDIs and potential toxicity studies on intended patient populations.</li> </ul>	<ul style="list-style-type: none"> <li>Most of HDIs evaluation are retrospective and are based on cases reports.</li> <li>Limited clinical trials and often carried out on healthy volunteers.</li> <li>Lack of monitoring of pharmacokinetic profiles of the herbal components.</li> <li>Lack of pharmacodynamics and potential toxicity in patient populations.</li> </ul>
Simulation and modeling	<ul style="list-style-type: none"> <li>PBPK modeling has been extensively applied to pharmacokinetic-based DDIs with complex mechanisms.</li> <li>Modeling and simulation are recommended by regulatory agencies<sup>5</sup>.</li> </ul>	<ul style="list-style-type: none"> <li>Only a few herbal products have been predicted of HDIs by PBPK modeling.</li> <li>Limited human pharmacokinetic data and lack of herbal standardization restrict the application of modeling and simulation on prediction of HDIs.</li> </ul>
Systematic reviews and databases	<ul style="list-style-type: none"> <li>A number of databases on DDIs analysis have been developed based on solid clinical evidence.</li> </ul>	<ul style="list-style-type: none"> <li>Few databases on HDIs have been established.</li> <li>No sufficient clinical data to support the effectiveness and safety of the combination.</li> </ul>



and small molecules, and has been successfully applied to quantitatively predict DDIs of CYP-modulating protein drugs (such as blinatumomab and sirukumab) and small molecule CYP substrates in patients<sup>169,170</sup>. On the other hand, pharmacokinetic interaction between two therapeutic biologics has seldom been reported. However, such pharmacokinetic DDIs may occur due to specific binding between two biologics. For example, palifermin is a truncated form of the endogenous fibroblast growth factor which contains the heparin-binding domains. Co-administration of palifermin with heparin was found to increase the systemic exposure to palifermin up to 5-fold<sup>171</sup>.

### 3.3.3. Pharmacodynamics-based interactions of therapeutic biologics

Comparing to the pharmacokinetics-based DDIs of therapeutics biologics, their pharmacodynamics-based DDIs are more commonly reported. A large volume of cases has demonstrated pharmacodynamic interactions among various hormones owing to their complex signaling networks<sup>159</sup>. For instance, insulin can interact with numerous drugs including hormones, antidiabetics, antibiotics, antipsychotics, etc.<sup>172</sup>. Recombinant growth hormones interact with small molecule hormones such as glucocorticoids, estrogens, thyroxine, etc.<sup>159</sup>. Although co-administration of biologics indicated for the same disease usually results in additive or synergistic efficacy, co-administration may also induce toxicity. Both anakinra and etanercept are approved for the treatment of rheumatoid arthritis. However, combined use of the two biologics led to severe adverse effects including increased risk of infection and increased neutropenia without significant improvement in therapeutic efficacy<sup>173</sup>.

### 3.3.4. Risk assessment for DDIs of therapeutic biologics

Due to the distinct pharmacokinetic and pharmacodynamic properties of therapeutic biologics, the classic approach for DDIs prediction for small molecules may not be applicable for therapeutic biologics. With the increase in therapeutic biologics in the market, it is critical to call for building strategies and regulations on the potential DDIs involving biologics. Based on the current findings on the major mechanisms for the pharmacokinetic-based DDIs of therapeutic biologics, assessment of the modulation of CYP activities and immunogenicity are recommended. In terms of pharmacodynamics-based DDIs, identification and monitoring of clinical endpoints relevant to both the efficacy as well as the adverse effects of therapeutic biologics is highly recommended.

### 3.4. Trends in microbiota mediated drug–drug interactions

Recent studies have indicated that the microbiota is a vital drug target in many disease treatments. Many therapeutics have great effects on altering the composition of the microbiota. As indicated in Fig. 2A, changes in microbiota in the gastrointestinal tract may influence the metabolism of co-administered drugs, leading to altered pharmacokinetics. Findings have shown that gut microbiota can mediate drug metabolism including reduction<sup>174</sup>, oxidation<sup>175</sup>, dehydroxylation, decarboxylation<sup>176</sup>, etc. DDIs between antibiotics and drugs that are metabolized by gut microbiota are commonly reported. Many antibiotics can disturb the PK of a co-administered drug by affecting the enzymatic activities and composition of gut microbiota<sup>177</sup>, leading to an altered therapeutic effect. For example, the coagulant drug sulfapyrazone can be metabolized to sulfapyrazone sulfide in the gut contents. It was found that the plasma pharmacokinetic profile of sulfapyrazone

and sulfapyrazone sulfide was changed in patients treated with the antibiotic metronidazole<sup>178</sup>. After reduction *via* azoreductases in gut microbiota, prontosil was metabolized to sulfanilamide, which exhibits potent antibacterial activities. In addition, it was noted in rats that the conversion of prontosil to sulfanilamide can be suppressed by antibiotics, leading to the reduced antibacterial effects<sup>174,179</sup>. Most recently, gnotobiotic mouse models and PBPK models have been established to untangle host and microbial contributions to the pharmacokinetic profile<sup>180</sup>. These novel experimental and computational strategies can be incorporated in future investigations on microbiota-mediated DDIs.

In addition to effects on pharmacokinetics, altered microbiota composition may also lead to pharmacodynamic changes in the concomitant drugs (Fig. 2B). It was noted that the presence of a certain type of bacteria may have an impact on chemotherapy and immunotherapy<sup>181,182</sup>. Clinical trials are currently conducted on microbiota interventions, such as probiotics and fecal microbiota transplant (FMT), to explore their influence on the efficacy and toxicity of co-administered chemotherapeutic agents, immunotherapeutic agents and anti-inflammatory drugs<sup>183</sup>. The potential benefits of probiotics and FMT to increase the efficacy of pembrolizumab in the treatment of PD-1 resistance patients<sup>184</sup> and to reduce the adverse effects of aspirin<sup>185</sup> and irinotecan<sup>186</sup> are currently under clinical investigation.

Besides well-known influences on the microbiota from antibiotics and probiotics, influences from other types of drugs or natural products are very limited. Although evidence of gut microbiota-mediated DDIs remain limited, the growing interest in microbiota will definitely provide a better understanding on their influence on the PK and pharmacodynamics of drugs. Nevertheless, the impact of herbal medicine on the gut microbiome is unavoidable, and such research is expected to provide more in-depth understanding on herb–drug interactions. In summary, in addition to consideration of classical PK and pharmacodynamic interactions, microbiota-mediated drug–drug/herb–drug interactions are expected to bring additional insight into their therapeutic effects.

### 3.5. Summary

Investigation of herb–drug interactions (HDIs) is often more complicated than that on DDIs, due to the complex herbal components and the batch-to-batch variation of herbal products. More pharmacokinetic and pharmacodynamic data on the bioavailable herbal components from clinical studies using standardized herbal products are warranted for better understanding of HDIs. With the increasing number of therapeutic biologics in the market, it is critical to build strategies and regulations on the potential DDIs involving biologics. Based on the current findings on pharmacokinetic- and pharmacodynamic-based DDIs of therapeutic biologics, assessments on the modulation of CYP activity and immunogenicity, and identification and monitoring of clinical endpoints of the therapeutic biologics is recommended. In addition to consideration of classical PK and pharmacodynamics interactions, microbiota-mediated HDIs/DDIs are expected to bring additional insight into their interactions. Novel experimental and computational strategies, such as gnotobiotic animal models and physiologically-based pharmacokinetic modeling can be incorporated in future investigations on microbiota-mediated HDIs/DDIs.

In summary, the incidence of interactions between various therapeutics is high in patients taking multiple drugs and dietary supplements. Although DDIs between small molecule drugs are



relatively well-characterized, other potential interactions are not fully explored. It is essential to develop efficient strategies for the investigation of the interactions between drugs and herbs, and between therapeutic biologics. Furthermore, the growing knowledge on the microbiota as therapeutic targets and as a site of drug metabolism leads us to pay more attention to microbiota-mediated interactions when examining potential DDIs and HDIs.

#### 4. Disease–drug interactions

Understanding disease–drug interactions is clinically important due to the risk of treatment failure and the incidence of adverse reactions. An accumulation of strong research evidence indicates that disease–drug and drug–disease interactions can have a profound effect on the response to a medication, yet most of the existing results are only from animal models. Moreover, there are differences between animal disease models and human diseases<sup>187</sup>. Differences between different species should be also taken into account. In recent years PBPK modeling has gradually been applied to the prediction of disease–drug interactions<sup>188,57</sup>. However, further clinical study or real-life experience is needed to justify results from PBPK modeling. Additionally, the potential mechanism of disease–drug interactions remains poorly characterized. Therefore, further studies are also needed to reveal the in-depth and comprehensive mechanism involved in disease–drug interactions.

In recent years, apart from the DDI, disease–drug interactions have attracted lots of attention due to their potential impact on efficacy and safety of clinical therapy. Disease–drug interactions mainly refer to the disease itself can lead to changes in PK and pharmacodynamics of drugs, and also include the influence of alteration of endogenous substrates related to metabolism on disease status. Both effects of disease on drug metabolism and effects of metabolism regulation on diseases have the potential to increase the risk of treatment failure and the incidence of adverse reactions<sup>189</sup>. Although there have been some reports published on

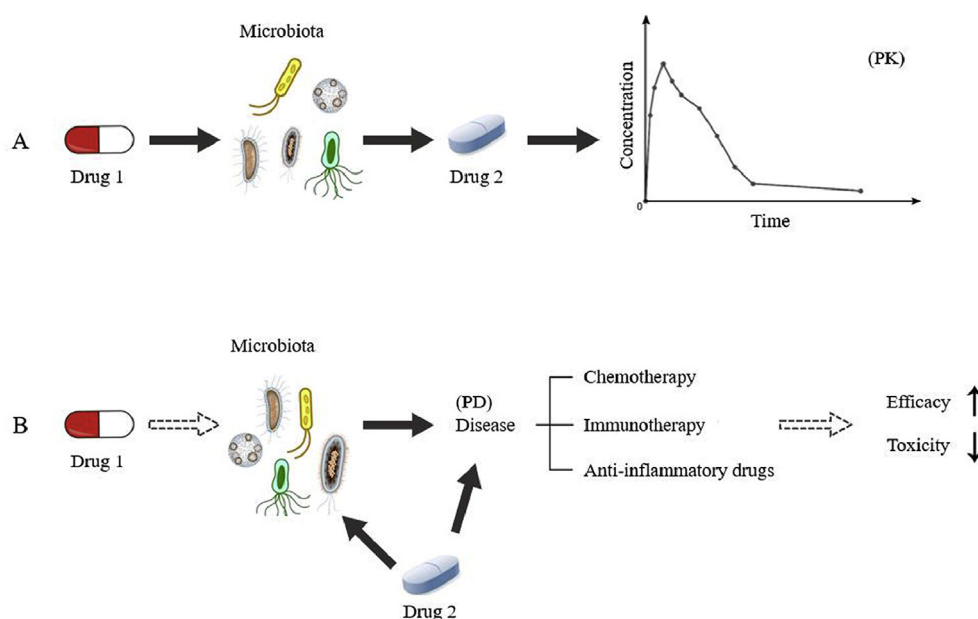
disease–drug interactions, there are still many unknown issues to be characterized. This review provides an update on the research on disease–drug interactions and offers an in-depth perspective on new strategies for the elucidation of disease–drug interactions.

##### 4.1. Effects of diseases on drug metabolism

Disease is a vital factor affecting clinical medication. Disease changes the PK of a drug by altering the ADME process; on the other hand, disease can also change the sensitivity of the body to drugs by altering the number of receptors and their function in organs. Clinical practice should take into account the effects of a disease on a drug for the best therapeutic outcome and to avoid serious adverse reactions by adjusting the dose, the interval of administration, and the route of administration, etc. Current progress on disease effects on drug metabolism are listed in Table 5.

##### 4.1.1. Effects of diabetes on drug metabolism

Diabetes mellitus, commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Diabetes mellitus is also a well-known risk factor for cardiovascular disease and atherosclerotic complications, especially coronary heart disease<sup>209</sup>. In recent years there have been many reports of the effect of diabetes on drug metabolism. Alterations in function and expression of ABC transporters at the blood–brain barrier in diabetes have been observed<sup>210</sup>; for instance, it was found that the uptake of vincristine by cultured rat brain microvessel endothelial cells incubated in diabetic rat serum were higher than uptake in nondiabetic rat serum, which was related to the impairment of P-gp function and expression at the blood–brain barrier of diabetic rats<sup>190</sup>. Moreover, in brain cortex, STZ-induced diabetes mellitus may induce an impairment of function and expression of BCRP. The uptake of prazosin and cimetidine, two typical substrates of BCRP, was significantly increased in diabetic rats compared to uptake in non-diabetic rats<sup>191</sup>. However, different from the



**Figure 2** Microbiota-mediated pharmacokinetic and pharmacodynamic interactions between different drugs. (The solid arrow indicates an effect supported by obtained evidence, and the dotted arrow indicates potential effects.)

impairment of function and expression of P-gp and BCRP, diabetes may enhance MRP2 function and expression in liver, kidney and intestine, which then leads to increased excretion of sulfo-bromophthalein (a substrate of MRP2) *via* the bile, urine and intestinal perfusate<sup>192</sup>. Atorvastatin is a substrate of OATP1B1, an influx transporter expressed on the sinusoidal membrane of hepatocytes. Recent studies found that diabetes mellitus could enhance the hepatotoxicity and decrease exposure to atorvastatin in rats partly through upregulating hepatic *Oatp2*<sup>193,194</sup>.

In addition to *Oatp2*, upregulation of hepatic *Cyp3a* also contributes to the decreased exposure to atorvastatin, simvastatin and simvastatin acid in diabetic rats<sup>193–195</sup>. Accumulated evidence shows that diabetes mellitus apparently alters the expression and activity of cytochrome P450 (CYP450) enzymes<sup>196,211</sup>. In diabetic rats, the AUC of theophylline was significantly smaller than that of normal rats because of significantly faster time-averaged total body clearance in diabetic rats, which was attributed to upregulated hepatic CYP1A2 and CYP2E1. Furthermore, diabetes mellitus could significantly increase exposure (area under the curve and peak concentration) to glibenclamide after oral administration. Data with hepatic microsomes suggested the impairment of glibenclamide metabolism and efflux in diabetic rats<sup>197</sup>. Accumulating evidence also has shown that diabetes increased the metabolism of CYP3A4 substrates by upregulating the function and expression of CYP3A4 in hepatic cells<sup>198</sup>. Interestingly, diabetes mellitus showed a tissue-specific effect on CYP3A expression and activity (induced in liver and inhibited in intestine), resulting in opposite pharmacokinetic behavior for verapamil after oral and intravenous administration to diabetic rats<sup>212</sup>. UGTs, the major phase II conjugation enzymes, can also be affected by diabetes mellitus. It was reported that the UGT1 family is adaptively upregulated in the diabetic gastrointestinal tract<sup>199</sup>. Given the essential regulatory role of the gastrointestinal site in drug disposition, such changes in UGTs may have an impact on the metabolism of therapeutic drugs and endogenous substrates.

#### 4.1.2. Effects of liver disease on drug metabolism

There is growing evidence to suggest that many hepatic diseases can affect drug metabolism. The effect of liver disease on drug metabolism is mainly due to the alteration of liver hemodynamics and activity of liver microsomal enzymes. Local and systemic liver injuries have a major effect on the expression and activity of DMEs in the liver<sup>213</sup>. For example, compared to control rats, there were significant changes in pharmacokinetic profiles after administrations of rhubarb anthraquinone-extracts in CCl<sub>4</sub>-induced liver-injury rats. The plasma concentrations of the four pharmacokinetic markers (Rhein, emodin, aloe-emodin, chrysophanol) of rhubarb anthraquinone extract increased, which indicated that their metabolism and excretion changed after liver injury<sup>200</sup>. Liver failure is often associated with hepatic encephalopathy, due to dyshomeostasis of the central nervous system (CNS). One study showed that the function and expression of P-gp and BCRP decreased, while the function and expression of MRP2 increased in the brain of acute liver failure (ALF) mice<sup>214</sup>. The attenuated function and expression of P-gp at the BBB might enhance phenobarbital distribution in the brain and increase phenobarbital efficacy on the CNS of ALF mice<sup>201</sup>. In addition, ALF could enhance oral plasma exposure of zidovudine in rats by down-regulation of hepatic UGT2B7 and intestinal P-gp<sup>202</sup>.

Fatty liver disease, also known as hepatic steatosis, is a condition where excess fat builds up in the liver. Previous research

showed that valproic acid with a high-fat diet-induced fatty liver could upregulate UGTs and was accompanied by the increased expression of CAR and PPAR $\alpha$ <sup>215</sup>. Further analysis revealed that liver disease in warfarin users was associated with a significant increase in the likelihood of hemorrhage<sup>216</sup>.

#### 4.1.3. Effects of heart failure on drug metabolism

Heart failure (HF) is considered an epidemic disease in the modern world affecting approximately 1%–2% of the adult population. Many CYP enzymes have been identified in the heart and their levels have been reported to be altered during HF. There is a great deal of discrepancy between various reports on CYP alterations during HF, likely due to differences in disease severity, the species in question and other underlying conditions. A recent review by Aspromonte et al.<sup>217</sup> has summarized a comprehensive modulation of cardiac CYP in patients with HF. In general, cardiac *CYP1B* and *CYP2A*, *CYP2B*, *CYP2J*, *CYP4A* and *CYP11* mRNA levels and related enzyme activities are usually increased in HF<sup>217,218</sup>. On the other hand, HF plays an important role in the down-regulation of hepatic CYP involved in drug metabolism through several mechanisms which include hepatocellular damage, hypoxia, elevated levels of pro-inflammatory cytokines, and increased production of heme oxygenase-1<sup>219</sup>. For example, the plasma concentrations of caffeine (CYP1A2 probe), mephenytoin (CYP2C19 probe), dextromethorphan (CYP2D6 probe) and chlorzoxazone (CYP2E1 probe) were significantly elevated in patients with congestive HF<sup>203</sup>. It was suggested that the doses of these CYP enzymes substrates should be decreased when used in patients with congestive HF.

#### 4.1.4. Effects of renal disease on drug metabolism

Evaluation of drug metabolism in patients with end-stage renal disease is important because these patients use a large number of medications and are at risk of adverse reactions and DDI. Previous studies found that end-stage renal disease patients had a 50% increase in the plasma warfarin *S/R* ratio relative to control subjects. This may be reflective of a selective decrease in hepatic CYP3A and CYP2C9 activity in renal failure<sup>204,205</sup>. Furthermore, results from a “cocktail” approach showed that the enzyme activities of CYP3A4 and CYP2C9 of patients with renal failure were selectively inhibited<sup>220</sup>. Therefore, if CYP3A4 and CYP2C9 substrates are used in patients with renal failure, the dose needs to be lowered. Although chronic renal failure (CRF) has been found to be associated with a decrease in liver CYP, the mechanism remains poorly understood. The *N*-demethylation of erythromycin was decreased by more than 35% ( $P < 0.001$ ) in hepatocytes incubated with serum from rats with CRF<sup>221</sup>. It is speculated that the mediator(s) of uremic serum may down-regulate the CYP of normal hepatocytes. In addition, a recent study investigated the effects of adenine-induced chronic kidney disease (CKD) in rats on the activities of some XMEs in liver and kidneys. It was found that the plasma theophylline concentration was significantly increased in rats with CKD<sup>206</sup>. Moreover, a reduced metabolism of midazolam could be observed in rats with acute kidney injury (AKI)<sup>207</sup>.

#### 4.1.5. Effects of sepsis on drug metabolism

Sepsis is the systemic inflammatory response syndrome caused by infection, which is a common complication following surgery, especially abdominal surgery, with higher mortality. It has been well documented that hepatocellular dysfunction occurs early in sepsis and contributes to multiple organ failure and ultimately death<sup>222</sup>. Among them, the effects of polymicrobial sepsis on the

**Table 5** Summary of the effects of specific diseases on drug metabolism.

Type of diseases	Affected drugs	Related mechanisms
Diabetes mellitus	<ul style="list-style-type: none"> <li>• Vincristine and other P-gp substrates: increased uptake<sup>190</sup>.</li> <li>• Prazosin, cimetidine and other BCRP substrates: increased uptake<sup>191</sup>.</li> <li>• Sulfobromophthalein and other MRP2 substrates: enhanced excretion<sup>192</sup>.</li> <li>• Atorvastatin, simvastatin: decreased exposure<sup>193–195</sup>.</li> <li>• Theophylline: increased metabolism<sup>196</sup>.</li> <li>• Glibenclamide: inhibited metabolism and decreased the efflux<sup>197</sup>.</li> <li>• CYP3A4 substrates: increased metabolism<sup>198</sup>.</li> <li>• UGT1 substrates: increased metabolism<sup>199</sup>.</li> </ul>	<ul style="list-style-type: none"> <li>• Impairment of P-gp function and expression.</li> <li>• Impairment of BCRP function and expression.</li> <li>• Induction of MRP2.</li> <li>• Upregulated OATP2, CYP3A.</li> <li>• Induction of CYP1A2 and CYP2E1.</li> <li>• Inhibition of CYP2C11 and BCRP.</li> <li>• Upregulated CYP3A4.</li> <li>• Upregulated UGT1.</li> </ul>
Liver disease	<ul style="list-style-type: none"> <li>• Rhein, emodin, aloe-emodin, chrysophanol: inhibited metabolism<sup>200</sup>.</li> <li>• Phenobarbital: enhance distribution<sup>201</sup>.</li> <li>• Zidovudine: inhibited metabolism and decreased the efflux<sup>202</sup>.</li> <li>• MRP2 substrates: enhanced efflux<sup>201</sup>.</li> </ul>	<ul style="list-style-type: none"> <li>• Inhibition of CYP and UGT metabolism.</li> <li>• Inhibition of P-gp and BCRP (brain).</li> <li>• Down-regulation of UGT2B7 and P-gp.</li> <li>• Induction of MRP2 (brain).</li> </ul>
Heart failure	<ul style="list-style-type: none"> <li>• Caffeine and other CYP1A2 substrates: inhibited metabolism<sup>203</sup>.</li> <li>• Mephenytoin and other CYP2C19 substrates: inhibited metabolism<sup>203</sup>.</li> <li>• Dextromethorphan and other CYP2D6 substrates: inhibited metabolism<sup>203</sup>.</li> <li>• Chlorzoxazone and other CYP2E1 substrates: inhibited metabolism<sup>203</sup>.</li> </ul>	<ul style="list-style-type: none"> <li>• Down-regulation of CYP1A2.</li> <li>• Down-regulation of CYP2C19.</li> <li>• Down-regulation of CYP2D6.</li> <li>• Down-regulation of CYP2E1.</li> </ul>
Renal disease	<ul style="list-style-type: none"> <li>• Erythromycin and other CYP3A substrates: inhibited metabolism<sup>204</sup>.</li> <li>• Warfarin and other CYP2C9 substrates: inhibited metabolism<sup>205</sup>.</li> <li>• Theophylline and other CYP1A1 substrates: inhibited metabolism<sup>206</sup>.</li> <li>• Midazolam and other CYP3A11 substrates: inhibited metabolism<sup>207</sup>.</li> </ul>	<ul style="list-style-type: none"> <li>• Inhibition of CYP3A.</li> <li>• Inhibition of CYP2C9.</li> <li>• Inhibition of CYP1A1.</li> <li>• Inhibition of CYP3A11.</li> </ul>
Sepsis	<ul style="list-style-type: none"> <li>• CYP1A1 substrates: inhibited metabolism<sup>208</sup>.</li> <li>• CYP1A2 substrates: inhibited metabolism<sup>208</sup>.</li> <li>• CYP2E1 substrates: inhibited metabolism<sup>208</sup>.</li> </ul>	<ul style="list-style-type: none"> <li>• Down-regulation of CYP1A1.</li> <li>• Down-regulation of CYP1A2.</li> <li>• Down-regulation of CYP2E1.</li> </ul>

activity and gene expression of hepatic microsomal CYP450 have attracted considerable attention due to their potential disease–drug interactions in clinical therapy. It has been reported that the major hepatic CYP isoforms CYP1A1, 1A2, 2B1, 2E1 were down-regulated during polymicrobial sepsis<sup>208,203–224</sup>. Moreover, results from mechanistic studies show that nitric oxide (NO) and the AhR play key potential roles in down-regulation of hepatic CYP during sepsis<sup>225,226</sup>. Therefore, treatment with pharmaceutical agents that regulate or are metabolized by CYP enzymes might be approached cautiously in the septic patient.

On the other hand, early and appropriate antimicrobial treatment is the predominant intervening measure to decrease patient mortality<sup>227</sup>. However, the pathophysiologic changes during sepsis such as systemic capillary leak syndrome, altered shift of body fluid and hypoalbuminemia can lead to changes in pharmacokinetics/pharmacodynamics (PK/PD) parameters such as apparent volume of distribution ( $V_d$ )<sup>228</sup> and clearance (CL)<sup>229</sup> that affect the achievement of PK targets and increase the risk of treatment

failure with routine dosing. In addition, it is likely to cause low blood protein symptoms in sepsis due to the increased capillary permeability, decreased hepatic albumin synthesis and a large number of infusions<sup>230</sup>. Therefore, the effect of hypoalbuminemia on antibiotic PK also cannot be ignored. It is crucial to reduce patient mortality by adjusting antimicrobial doses and improving drug infusion to optimize antimicrobial therapy according to the characteristics of PK/PD during sepsis.

#### 4.2. Effects of endogenous metabolism mediated by nuclear receptors on diseases

In recent years the regulation of endogenous metabolism mediated by nuclear receptors on diseases has received increasing attention with improvements in bioanalytical technology, especially the intervention of the various “omics”. Among them, PXR and CAR are two closely related and liver-enriched nuclear hormone receptors originally defined as xenobiotic receptors. However, an

increasing body of evidence suggests that PXR and CAR also have endobiotic functions that impact glucose and lipid metabolism, as well as the pathogenesis of metabolic diseases. PXR and CAR not only regulate the transcription of drug-metabolizing enzymes and transporters, but also orchestrate energy metabolism and immune responses<sup>231</sup>. The cutting-edge investigations on these aspects are briefly shown in Table 6.

A recent study revealed that PXR ablation inhibited high-fat diet-induced obesity, hepatic steatosis, and insulin resistance<sup>232</sup>. These results may help to establish PXR as a novel therapeutic target, and PXR antagonists may be used for the prevention and treatment of obesity and type 2 diabetes. PXR was also reported to play a vital role in maintaining biliary bile acid homeostasis by regulating the biosynthesis and transport of bile salts<sup>233</sup>. Activation of the PXR pathway was associated with decreased lithocholic acid-induced cholestasis in mice<sup>241</sup>. PXR may be developed as a therapeutic target for cholesterol gallstone disease. Interestingly, study has revealed a function of PXR in enlarging liver size and changing liver cell fate by activation of the yes-associated protein (YAP) signaling pathway. This has implications for understanding the physiological functions of PXR<sup>242</sup>. In addition, PXR plays an important endobiotic role in adrenal steroid homeostasis. Activation of PXR markedly increased plasma concentrations of corticosterone and aldosterone<sup>234</sup>. These results suggest that PXR is a potential endocrine disrupting factor that may have broad implications in steroid homeostasis and drug–hormone interactions.

CAR has also been increasingly appreciated for its endobiotic functions in influencing glucose and lipid metabolism, with dysregulation implicated in two of the most prevalent metabolic disorders, obesity and type 2 diabetes<sup>243</sup>. Further study found that CAR suppresses hepatic gluconeogenesis by facilitating the ubiquitination and degradation of PGC1 $\alpha$ <sup>244</sup>. Given the metabolic benefits of CAR activation, CAR may represent an attractive therapeutic target to manage obesity and type 2 diabetes.

Nonalcoholic steatohepatitis (NASH) is common and medically significant because it is closely related to metabolic syndrome and has the potential to progress into the more harmful cirrhosis. Emerging evidence points to an important function of AhR in the uptake of fatty acids in the liver and the pathogenesis of fatty liver disease<sup>236</sup>. Activation of the AhR sensitizes mice to NASH by deactivating mitochondrial sirtuin deacetylase Sirt3<sup>245</sup>. These results suggest that the use of AhR antagonists might be a viable approach to prevent and treat NASH.

LXRs are known as sterol sensors that impact cholesterol and lipid homeostasis, as well as inflammation. The hepatic functions of LXRs are well documented and the pathophysiological role of LXRs was uncovered progressively in recent years. Activation of LXR prevents lipopolysaccharide-induced lung injury by regulating antioxidant enzymes and the implication of this regulation is pulmonary tissue protection<sup>237</sup>. Moreover, a recent study demonstrated that activation of LXR attenuates OA-induced acute respiratory distress syndrome by attenuating the inflammatory response and enhancing antioxidant capacity<sup>238</sup>.

FXR, a nuclear receptor mainly expressed in enterohepatic tissues, is a master regulator for bile acid, lipid and glucose homeostasis<sup>246</sup>. Emerging evidence indicates that restoration of FXR protein levels may represent a new strategy for enterohepatic and metabolic diseases. Hepatitis B virus X protein (HBx) is a hepatitis B virus protein that has multiple cellular functions, but its role

in the pathogenesis of hepatocellular carcinoma (HCC) has been controversial. It was reported that transactivation of FXR by full-length HBx may represent a protective mechanism to inhibit HCC<sup>247</sup>. Additionally, FXR antagonism was also reported to be pivotal in attenuating obstructive cholestasis in bile duct-ligated mice<sup>235</sup>. These results suggest that FXR may be developed as a therapeutic target for cholesterol gallstone disease.

The tumor suppressor p53 is traditionally recognized as a surveillance molecule to preserve genome integrity. Recent studies have demonstrated that it contributes to metabolic diseases. It was found that the activation of p53 participated in promoting bile acid disposition and alleviating cholestatic syndrome by up-regulating the expression of *Cyp2b10*, *Sult2a1* and *Abcc2/3/4*, which provides a potential therapeutic target for cholestasis<sup>235</sup>. In addition, p53 could attenuate acetaminophen-induced hepatotoxicity by regulating the CYPs, SULTs and MRPs, which provides a potential new therapeutic target for APAP-induced liver injury<sup>248</sup>.

Metabolism regulation mediated by downstream targets of the above transcriptional factors may also play an important role in diseases. For example, NAD(P)H: quinone oxidoreductase 1 (NQO1) has been reported to be a prognostic biomarker and a promising therapeutic target for patients with NSCLC due to its frequent overexpression and significantly increased activity in NSCLC. It was found that depleting tumor-NQO1 potentiates anoikis and inhibits the growth of NSCLC<sup>239</sup>. Furthermore, recent results from a metabolomics analysis have revealed that inhibition of cell proliferation upon NQO1 depletion was accompanied by suppressed glycometabolism in NQO1 high-expression human NSCLC A549 cells. Also, NQO1 depletion significantly decreased the gene expression of hexokinase II<sup>240</sup>.

#### 4.3. Summary

Understanding disease–drug interactions is clinically important due to the risk of treatment failure and the incidence of adverse reactions. An accumulation of strong research evidence indicates that disease–drug and drug–disease interactions can have a profound effect on the response to a medication, but most of the existing results are only from animal models. In recent years, PBPK modeling has also gradually been applied to the prediction of disease–drug interactions<sup>57,188</sup>. However, further clinical study or real-life experience is certainly needed to justify the results from PBPK modeling. Additionally, the potential mechanisms of disease–drug interactions are not well-characterized. Therefore, further studies are needed to reveal the in-depth and comprehensive mechanism involved in disease–drug interactions.

### 5. Mathematical modeling

The application of mathematical modeling to problems in PK has a rich history in the form of pharmacokinetic modeling to explore how simulation can be used to improve our understanding of common issues not readily addressed in human pharmacology<sup>249</sup>. Animal models are mainly used in experimental physiology, experimental pathology and experimental therapeutics, especially in the study of new drugs. In the earliest stage of drug discovery/development, various cell-based models and animal models were used for the prediction of human PK and toxicokinetics<sup>250</sup>. In this section, the current status and future challenges on PBPK modeling and animal models are summarized.



### 5.1. The current status and challenges of PBPK modeling

As early as 1937<sup>251</sup> physiological parameters were introduced into pharmacokinetic parameter estimation. The term PBPK model appeared in 1977<sup>252</sup>. Although the PBPK method was proposed a long time ago, it was applied to support new drug development in the last decade since the mechanism of drug metabolism and transport gained clarity. Two known milestones of extensive application for industry are: 1) A PBPK review team was set up in the office of clinical pharmacology (CDER/FDA, US) because of increasing numbers of PBPK submissions in 2013<sup>253</sup>; 2) PBPK guidance was released by FDA<sup>254</sup> and EMA<sup>255</sup> respectively during 2016–2018. A total of 217 PBPK submissions were reviewed by the FDA in 2016<sup>256</sup>. As one of the four major pharmacometric research methods<sup>257</sup>, the strategy of waiving clinical trials through PBPK study has been extensively accepted in western society and is gradually being accepted in China.

#### 5.1.1. Basic concepts of PBPK

PBPK can be utilized to mechanistically understand and predict *in vivo* pharmacokinetic characteristics from a whole body perspective by integrating system-specific parameters (such as physiological parameters), drug-specific parameters (such as physical–chemical and mechanistic pharmacokinetic data), and specific PBPK model structure<sup>258</sup>. It can quantitatively describe drug concentration kinetics in the blood and each tissue through a series of mathematical differential equations, which allows it to accurately predict target tissue drug concentration as well as to understand drug absorption, distribution, metabolism, elimination, and transportation (ADMET) processes. Because it incorporates system-specific parameters into equations of each tissue, it can also be used to predict drug concentration in tissues under different scenarios, such as co-administration of enzyme inhibitor or in a specific population (hepatic- or renal-impaired patients, pediatrics, or elders), which could support new drug development strategy, clinical trial design, and improved clinical development efficiency.

#### 5.1.2. PBPK in drug development

**5.1.2.1. PK drug–drug interaction study.** As of August 2016, 60% of PBPK study cases submitted to FDA were related to drug–drug interactions (DDI)<sup>256</sup>. Among the three predominant DDI mechanisms, enzyme-<sup>259</sup>, transporter-<sup>260</sup>, and disease-mediated DDI<sup>261,262</sup>, enzyme-mediated DDI cases showed the best predictive performance in PBPK. Hsueh et al.<sup>259</sup> summarized 104 publications with DDI predictions, a total of 126 and 360

cases were reported for drug as metabolic “victim” and “perpetrator” respectively. The predictive performance of CYP3A- and CYP2D6-mediated DDI was found to be the best for new drugs as victim using the PBPK method. Two enzymes are involved in metabolism of large proportion of marketed drugs and well-established probe perpetrators are available<sup>256,263</sup>. The predictive performance was poorer for new drugs as perpetrator<sup>259</sup>. In order to accurately predict the quantitative effects of an enzyme inhibitor<sup>264</sup> or inducer<sup>265</sup> on a substrate, the FDA suggested the following study strategy<sup>256,266</sup>: a) Develop an initial PBPK model of enzyme–substrate based on *in vitro* data followed by verification using human single-dose PK data; b) develop a PBPK model of inducer or inhibitor and validate its enzyme modulation effect using *in vivo* (or literature data) data; c) predict the effect of inhibitor/inducer on substrate PK characteristics in humans using the PBPK model, which will support DDI study strategy or clinical trial design, especially for the dose selection; d) if a dedicated DDI was required and conducted, then the initial PBPK model will be verified and modified based on observed DDI data; e) predict other untested scenarios and validate dose selection. Following this strategy, predictive performance was summarized in report published by Hsueh et al.<sup>259</sup>. As stated in submitted cases to the FDA, AUCR or  $C_{\max}$ R (ratio of AUC or  $C_{\max}$ ) was estimated within the range of (0.80, 1.25) and (0.50, 2.00) for higher than 73% and 77% cases respectively. Although overall DDI of CYP-mediated interactions could be estimated well, prediction of time-dependent DDI and intestinal enzyme-mediated DDI was still challenging<sup>256</sup>.

Because tissue concentration can indicate efficacy or safety better than plasma concentration, it is more important to be predicted, especially for the drugs with a “disconnected” concentration in tissues compared to plasma concentration, which may be caused by significantly different distribution through transporters<sup>267</sup>. Unfortunately, the best prediction method theoretically, the PBPK method, showed worse predictive performance for transporter-mediated DDI compared to that of enzyme-mediated DDI, which was due to ubiquitous tissue distribution, unique cellular localization, and competing active and passive processes<sup>268</sup>. Furthermore, the lack of knowledge pertaining to disease- or population-specific factors makes PBPK more challenging for transporter-mediated DDI prediction. In order to accurately predict unbound and intra/subcellular drug concentrations while considering the role of a transporter, selecting appropriate *in vitro* (such as imaging) and *in vivo* experimental methods to determine tissue concentration followed by verification of PBPK model in animals may be helpful<sup>267</sup>. Recently,

**Table 6** Summary of endogenous substances related to diseases and nuclear receptors.

Endogenous substance	Diseases involved	Related nuclear receptor or protein
Glucose and lipid	• Obesity and type 2 diabetes <sup>232</sup>	• PXR, CAR
Biliary bile acid	• Cholesterol gallstone <sup>233,234</sup>	• PXR, FXR
	• Cholestatic syndrome <sup>235</sup>	• P53
Corticosterone and aldosterone	• Steroid dyshomeostasis <sup>234</sup>	• PXR
Fatty acids	• Nonalcoholic steatohepatitis <sup>236</sup>	• AhR
Sterol	• Lung injury <sup>237</sup>	• LXR
Oleic acid	• Acute Respiratory Distress Syndrome <sup>238</sup>	• LXR
Hexokinase II	• Non-small cell lung cancer <sup>239,240</sup>	• NQO1



disease-mediated DDI received greater attention, especially for renal impairment affecting liver enzymes<sup>262</sup>. However, research on disease-mediated DDI are limited, and few PBPK cases to predict this kind of DDI have been reported<sup>269,270</sup>, and so further research to uncover the rationales behind of disease and physiological parameters is needed.

**5.1.2.2. Specific population study.** One of the most known characteristics of the PBPK model is that it can integrate drug-specific and system-specific parameters, which includes age, gender, disease status, and specific physiological status. This characteristic allows us to predict PK exposure changes by mechanistically changing specific parameters according to the different populations, such as pediatric, elderly, and in patients with hepatic or renal impairment. However, accurate prediction for these specific populations is still quite challenging because changes in system-specific parameters generally are not available or quantified accurately<sup>271</sup>. The FDA and other scientists summarized PBPK prediction strategy in patients with renal impairment<sup>272</sup> or hepatic impairment<sup>273</sup>, in the elderly<sup>274</sup>, pediatric<sup>275</sup>, fetal<sup>276</sup>, and pregnant patients<sup>277</sup> but, because of the above limits, these predictions could be utilized only to aid in clinical trial design in these specific populations rather than to waive these dedicated clinical trials without any verification in these specific populations.

**5.1.2.3. Generic drug development.** In comparison to the *in vitro*–*in vivo* correlation (IVIVC) method the PBPK method was advantageous because it could identify the contribution of penetration, intestinal metabolism and transport to the absorption–drug concentration–time curve. Therefore, PBPK analysis can estimate *in vivo* dissolution characteristics more accurately, which will be useful to guide drug development<sup>278,279</sup>. Therefore, the US FDA continuously held modeling and simulation workshops to make PBPK methods more useful in generic drug development<sup>280–282</sup> as well as suggested a research strategy for industry<sup>283,284</sup>. Additionally, physiologically-based oral absorption modeling can be utilized to guide Quality by Design (QbD) and predict food effects, effect of acid-reducing agents, SUPAC activities, and to influence label language. Although it was potentially powerful, its application is still limited because of physiological information missing in PBPK system models. The European OrBiTo (Oral Biopharmaceutical Tool) Project results showed that less than 50% of drugs could receive 2-fold error prediction performance using the PBPK modeling method<sup>285</sup>. Modified *in vitro* experiment data with more similarities to *in vivo* status and accurate physiological parameters affecting the rate-limiting absorption process may be able to improve its predictive performance.

**5.1.2.4. Other applications and trends of PBPK modeling methods.** In addition to the above applications, the PBPK modeling method also could be used to predict first-in-human PK profiles<sup>286</sup>. It may be helpful for those drugs with nonlinear metabolism characteristics. Recently, a semi-PBPK model (or minimal PBPK model)<sup>287,288</sup> was reported to extensively survey human biologics PK profiles to assess the predominant clearance site and dynamically describe system plasma concentrations and two other virtual compartments, lumped tissues with continuous and fenestrated vascular endothelium. This semi-PBPK model structure could allow investigators quickly estimate PBPK parameters using system drug concentrations considering drug–

receptor binding in systems as well as in tissues, as described in two recent reviews<sup>289,290</sup>.

The PBPK modeling method is not an independent modeling method, and sometimes it is better to be integrated with other modeling methods for better results. In order to understand PK characteristics in mechanism, allometric scaling<sup>291</sup> and *in vitro*–*in vivo* extrapolation methods<sup>292</sup> can also be used to analyze preclinical data and compare the results with human data, which can provide more key information from different angles to develop a PBPK model more accurately indicating the real disposition process in humans<sup>293</sup>. Taking advantage of the PBPK ability to predict drug tissue concentration, a PBPK-PD model could be developed to capture pharmacodynamic characteristics in a more accurate way with more understanding of the mechanism<sup>294,295</sup>, which is helpful for those drugs with significantly inconsistent exposure between system and targeted tissues. For a new moiety entity clinical development, verification of an established PBPK model based on human data with the specific ADMET mechanism is required, which may need an additional clinical trial. Recently, global development is going to become routine strategy, and ethnic differences in PK characteristics will be important. Therefore, PBPK could support evaluation of ethnic differences by its unique contribution to the mechanistic understanding<sup>296</sup>. Because population PK (PopPK) is routinely used to identify the key factors affecting PK profiles followed by quantifying these key factors, a PopPK study could verify PBPK simulations under some extreme scenarios, which may allow sponsors to waive some dedicated clinical trials (PBPK-PopPK strategy)<sup>297</sup>. Under many scenarios, we only pay attention to drug concentration in tissues related to PK, PD, or safety characteristics, so we don't need to accurately capture drug kinetics in other tissues. Therefore, in order to increase parameter reliability without a decrease in PBPK power, we could shrink the typical PBPK model integrating each tissue in humans to a semi-PBPK model integrating necessary target tissues and replace other tissues with one or two compartments.

Along with the coadministration of herbal or natural products, the potential herb–drug interaction is gaining increasing attention, and can be predicted using a PBPK modeling method. But accurate prediction of herb–drug interactions is still a challenging mission because of the complex composition and relatively limited knowledge of individual constituents that produce the interactions. A feasible procedure is to firstly identify the major constituents followed by compound–compound interaction prediction as previous introduced<sup>158,298</sup>. The major concern with this procedure is to prove that the interaction of major constituents is similar to that of the whole herb.

## 5.2. Summary

In summary, PBPK can be utilized to mechanistically understand and predict *a priori in vivo* pharmacokinetic characteristics from a whole body perspective by integrating system-specific and drug-specific parameters. PBPK modeling has been routinely conducted for new entities to illustrate pharmacokinetic characteristics when drug–drug interactions happen or when dosing in specific populations needs optimization. The predictive performance of CYP3A- and CYP2D6-mediated DDI was found to be best for new drugs as victim using PBPK method, which could be applied to waive part of clinical trial. Due to unclear changes in transporter-mediated mechanism and system-specific parameters in specific populations, PBPK modeling power is limited to supporting clinical trial design.

## 6. Novel animal models for DMPK studies

Animal models are mainly used in experimental physiology, experimental pathology and experimental therapeutics, especially in the study of new drugs. In the earliest stage of drug discovery/development, various cell-based models and animal models were used for the prediction of human PK and toxicokinetics<sup>250</sup>. The common laboratory animals for DMPK include rats, rabbits, dogs, monkeys, etc. However, with the development of gene editing technology, animal models of special ADME genes are needed to better study the mechanisms of DMPK, including the metabolic pathway and its regulatory mechanism.

### 6.1. Conventional transgenic animal models for DMPK research

Traditional animal models are constructed by homologous recombination in embryonic stem cells. This method implements foreign gene knock-in, but the recombination efficiency is very low, and the recombinant site has certain randomness<sup>299</sup>. In 2009, the discovery and application of nucleic acid engineering enzymes greatly advanced gene knock-in technology<sup>300</sup>. Zinc-finger nucleases (ZFNs) are the first nucleic acid engineering enzymes to be discovered<sup>300</sup>. They cleave DNA at specific sites to form double-strand breaks (DSBs), which are then repaired by cell homology and used as templates by exogenous donor DNA. The repair of DSBs result in knocking out the foreign gene<sup>300</sup>. Another engineering nuclease that was subsequently discovered for gene editing is transcription activator-like effector nucleases (TALENs)<sup>300,301</sup>. Since the 1990s, *Cyp* knockout (KO) mice have been successfully constructed using gene KO techniques, such as *Cyp1a2*, *Cyp2e1*, *Cyp2c9*, *Cyp3a4* and *Cyp2d6*<sup>302–305</sup>. In recent years some of mouse models have been used to study the DMPK of drugs under specific *Cyp* knockout conditions. To overcome the differences in subtype composition, protein expression, catalytic activity and substrate specificity between mouse and human CYP enzymes, scientists have built humanized animal models to better evaluate drug metabolism characteristics of human CYPs. For example, in 2007 humanized *Cyp1a1/2* mice were constructed for a toxicology study<sup>306</sup>. Humanized *Cyp2c19* mice for drug metabolism<sup>307</sup>, humanized *Cyp3a4* mice for drug interactions<sup>308</sup>, and humanized *Cyp2d6* mice for drug interactions<sup>309</sup> were reported in 2008, 2011 and 2012, respectively. In 2012, *Cyp2c* knockout mice and *Cyp2c9* humanized mice were generated for drug metabolism and drug interaction studies<sup>304</sup>. In 2015, humanized *Cyp2b6* mice were also constructed for drug metabolism<sup>310</sup>.

Both *Cyp* gene KO and humanized mouse models have been constructed by traditional knockout techniques, *i.e.* homologous recombination of foreign DNA fragments with genes of the same or similar sequence in the host genome, thus replacing the corresponding gene sequences in the genome of the recipient cells and integrating them into the host. In the cell genome, the key technologies of this method include the acquisition of embryonic stem cells, the design of target, and the screening of embryonic stem cells. Homologous recombination is time-consuming, costly, as well as inefficient in gene editing, and may lead to adverse mutations. As it is difficult to obtain and culture embryonic stem cells in rats, the construction and application of knockout or knock-in rat models have lagged behind the mouse models. Rats are a rodent model animal widely used in DMPK and have many advantages over mice, such as larger size, easy manipulation, high tolerance to blood volume loss and large sample size. Moreover,

rats in certain physiological and pathological states such as diabetes and breast cancer, are closer to humans than mice<sup>311,312</sup>. Therefore, it is particularly urgent to construct novel rat models of DMPK-related genes through KO and humanization.

### 6.2. Novel CRISPR/Cas9-based animal models for DMPK research

The clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated 9 (Cas9) system, as the third generation of artificial nuclease technology, provides a promising tool for genetic engineering. It offers an efficient approach to develop genetically modified animal models and a potential strategy for targeted gene therapies. The CRISPR/Cas9 system allows simultaneous digestion of multiple targets at multiple sites in the same cell, making it possible to knock out or knock in multiple genes. CRISPR/Cas9 as a new gene editing technology has many characteristics and advantages, including high targeting accuracy, simultaneous knockout of multiple sites of target genes, simplicity of operation and no species restriction. In recent years, CRISPR/Cas9 has been applied to the study of drug absorption, disposition, metabolism and excretion, as well as the preparation of ADME animal models.

Today CRISPR/Cas9 technology enables DMPK scientists to develop better and more predictable ADME models *in vitro* and *in vivo*, especially to study ADME genes that have not been fully explored previously. Most published papers of CRISPR/Cas9-mediated ADME describe CYP drug metabolic enzymes and ABC drug transporters. For example, in 2016, the rat *Cyp2d* gene locus (containing *Cyp2d1-5*) was knocked out and replaced with human *CYP2D6* in Wistar rats, but a functional characterization was not reported<sup>313</sup>. In the same year the rat *Cyp2e1* gene was knocked out in Sprague Dawley rats, and the KO rats were physiological normal and lost the expression and function of the CYP2E1 enzyme<sup>314</sup>. In 2017, *Cyp3a1* and *Cyp3a2* double KO rats were generated by CRISPR/Cas9 technology<sup>315</sup>, and *Cyp2c* (*Cyp2c6*, *Cyp2c11* and *Cyp2c12*) genes were also knocked out in rats<sup>316</sup>. Finally, *Cyp2c11* gene was knocked out in Sprague Dawley rats<sup>317</sup>. *In vitro* and *in vivo* metabolic studies of the CYP substrates indicated that the target CYP isoform was functionally inactive in all KO rats<sup>314,315</sup>. It should be noted that KO models resulted in the compensatory regulation of other CYP isoforms involved in drug metabolism<sup>314,315</sup>. However, the potential mechanisms of these compensatory changes remain unclear. In addition, these KO models showed some differences, such as changes in serum testosterone concentrations<sup>315</sup> or alkaline phosphatase<sup>314</sup>. Some of these differences can be attributed to the deficiency of CYP functions, such as CYP3A-mediated testosterone metabolism. Therefore, these physiological changes in KO rats should be considered when comparing ADME data from KO models with data from wild-type rats. In addition to the rat KO models, a *Cyp2b9/10/13* KO mouse model was also generated *via* CRISPR/Cas9 technology<sup>318</sup>. It is interesting that there were no significant compensatory changes in other CYP isoforms in *Cyp2b* KO mice, which may be due to low CYP2B hepatic expression, especially in male mice<sup>318</sup>. In 2019, a novel MDR1 (*Mdr1a/b*) double-knockout rat model was generated in Sprague Dawley rats by the CRISPR/Cas9 technology<sup>319</sup>. The loss of MDR1 function significantly increased digoxin uptake in *Mdr1a/b*<sup>-/-</sup> rats. The MDR1 KO rat model is of great significance to study the function of MDR1 in drug transport, toxicity and drug resistance.

### 6.3. Summary

In summary, genome editing based on CRISPR/Cas9 has been identified as a breakthrough technology in constructing animal models. Novel animal models are not only conducive to the basic research of human diseases, but also can be used to study the molecular mechanisms of drug pharmacodynamics, toxicity and clinical use. Furthermore, DMPK animal models will promote the study of DMPK mechanisms and strengthen the relationship between drug metabolism and pharmacology/toxicology. For example, the potentials and mechanisms of DDI between erlotinib and docetaxel was studied by using *Cyp3a1/2* KO rats<sup>320</sup>. Docetaxel significantly increased the maximum concentration and systemic exposure of erlotinib in wild type (WT) rats, but the DDI was significantly attenuated in *Cyp3a1/2* KO rats, suggesting that the CYP3A plays the perpetrating role of docetaxel on erlotinib.

## 7. Non-classical xenobiotic metabolic pathways

Drug metabolism or drug biotransformation is the process by which xenobiotics are enzymatically modified to make them more readily excretable and eliminate pharmacological activity. Drug metabolism is the prominent process in drug disposition. Understanding the metabolic fate and the corresponding enzymes are important with regard to metabolite toxicity and drug–drug interaction risks. Detailed data from drug metabolism studies aid in the drug clinical practice and drug design and modification. Over the past decades the basic mechanism and rules of drug metabolism, especially mediated by CYP, have been clarified. The strategies and approaches used for drug metabolism investigations have come to maturity and industrialization. Recently, with the rapid development of the separation technology and qualitative techniques, such as IMS-TOF/MS or novel 2D NMR technology, and the considerable amount of attention directed at non-CYP enzymes, several undesirable drug metabolites have been identified, and novel metabolic reactions were discovered. Some outwardly rational reactions are newly described based on the novel understandings of the mechanism underlying common biotransformations. This section briefly reviews a series of cases of novel metabolic reactions and pathways to provide readers new insights into investigations on drug metabolism.

### 7.1. Oxidative pathways

Oxidative pathways, including *sp*<sup>3</sup>-hybridized *C*-hydroxylation, unsaturated *C*-oxidation, *N*-dealkylation/deamination, *O*-dealkylation, *S*-dealkylation, *N*-oxidation, *S*-oxidation, and oxidative cleavage of esters and amides classified by functional groups are the most common biotransformations. In recent years some unexpected oxidative reactions or pathways have been reported.

Aromatic ring-containing drugs are most common and generally metabolized by P450-mediated  $\pi$ -electron oxidations to form an arene-epoxidized intermediate. The latter undergoes a hydride shift spontaneously to produce stable phenol metabolite(s). However, for some specific structures, unstable epoxides are preferentially attacked by nucleophilic substances, thereby leading to reactive intermediate-related covalent attachments. For example, for cocaine, the covalent adducts of biological thiols are first characterized<sup>321</sup>. *In vitro* investigations revealed that CYP1A2, 2C9, and 2D6 catalyze the formation of a reactive epoxide intermediate from the oxidation of the cocaine phenyl

moiety (Fig. 3A). Although an aryl moiety is generally considered a stable functional group, epoxide ring opening is attacked by nucleophilic thiols, such as *N*-acetylcysteine or glutathione, for cocaine.

Carbon–carbon cleavage and formation reactions are rare in xenobiotic metabolism. Recent studies have focused on the roles of flavin-containing monooxygenases (FMOs) to catalyze unexpected Baeyer–Villiger oxidations, which is a kind of carbon–carbon bond cleavage reaction. E7016, a potential anticancer agent with a 4-hydroxypiperidine moiety was confirmed to be a substrate of FMO5<sup>322</sup>. The generation of the major ring-opened hydroxyl-carboxylate metabolite was proposed by a three-step reaction, as follows: dehydrogenation of the secondary alcohol on the parent drug to form piperidine-4-one, followed by insertion of an oxygen atom to form a lactone *via* the Baeyer–Villiger oxidation, and further CEs-mediated hydrolysis. Recently, the 2,3-dihydropyridin-4-one (DHPO) ring in MRX-I (an analog of the antibiotic linezolid) was also reported to undergo a similar carbon–carbon cleavage reaction in humans<sup>323</sup>. However, different from piperidine-4-one, Baeyer–Villiger oxidation of the DHPO ring forms an enol lactone and is further hydrolyzed to an enol, which can be transformed to an aldehyde intermediate by enol–aldehyde tautomerism. The aldehyde intermediate underwent either oxidation catalyzed by short-chain dehydrogenase, aldehyde ketone reductase, and aldehyde dehydrogenase (ALDH) or reduction mediated by ALDH to generate the observed directed DHPO ring-opening metabolites MRX459 or MRX455-1, respectively (Fig. 3B). H<sub>2</sub><sup>18</sup>O experiments were conducted to elucidate the mechanism underlying the formation of the two metabolites.

### 7.2. Reductive metabolic pathways

The majority of *in vivo* biotransformations are oxidation, while reductive reactions preferentially occur in anaerobic or low-O conditions. A considerable number of the same enzymes that catalyze oxidative metabolism, such as P450s, aldo-keto reductase, carbonyl reductase, xanthine oxidase, aldehyde oxidase, and quinone oxidoreductase, can also be involved in reductions. Under the catalysis of some specific enzymes or the involvement of reducing agents, some uncommon reductive metabolic pathways are observed.

NADPH-cytochrome P450 reductase (POR) and cytochrome-b5 is crucial for P450 electron transporter chain integrity because they donate electrons to P450s from NADPH. Thus, most marketed recombinant P450 enzymes generally contain cytochrome-b5 and POR to enhance their oxidative efficiencies. In some cases, POR alone can also catalyze one-electron reduction, such as with aristolochic acid<sup>324</sup>. Another reported substrate of POR is an aldehyde intermediate (M-CHO) that is formed during the metabolism of imrecoxib, which is a moderate COX-2 inhibitor<sup>325</sup>. POR expresses dual effects on further M-CHO metabolism, namely oxidation to form carboxylic acid metabolite (M2) and unexpected reduction to form a hydroxymethyl metabolite (M1), by donating electrons to P450s or competitively to the substrate, respectively (Fig. 4A). The two opposite metabolic pathways, especially M-CHO reduction, led to an underestimation of the amount of M2 in static *in vitro* incubations.

### 7.3. Hydrolysis pathways

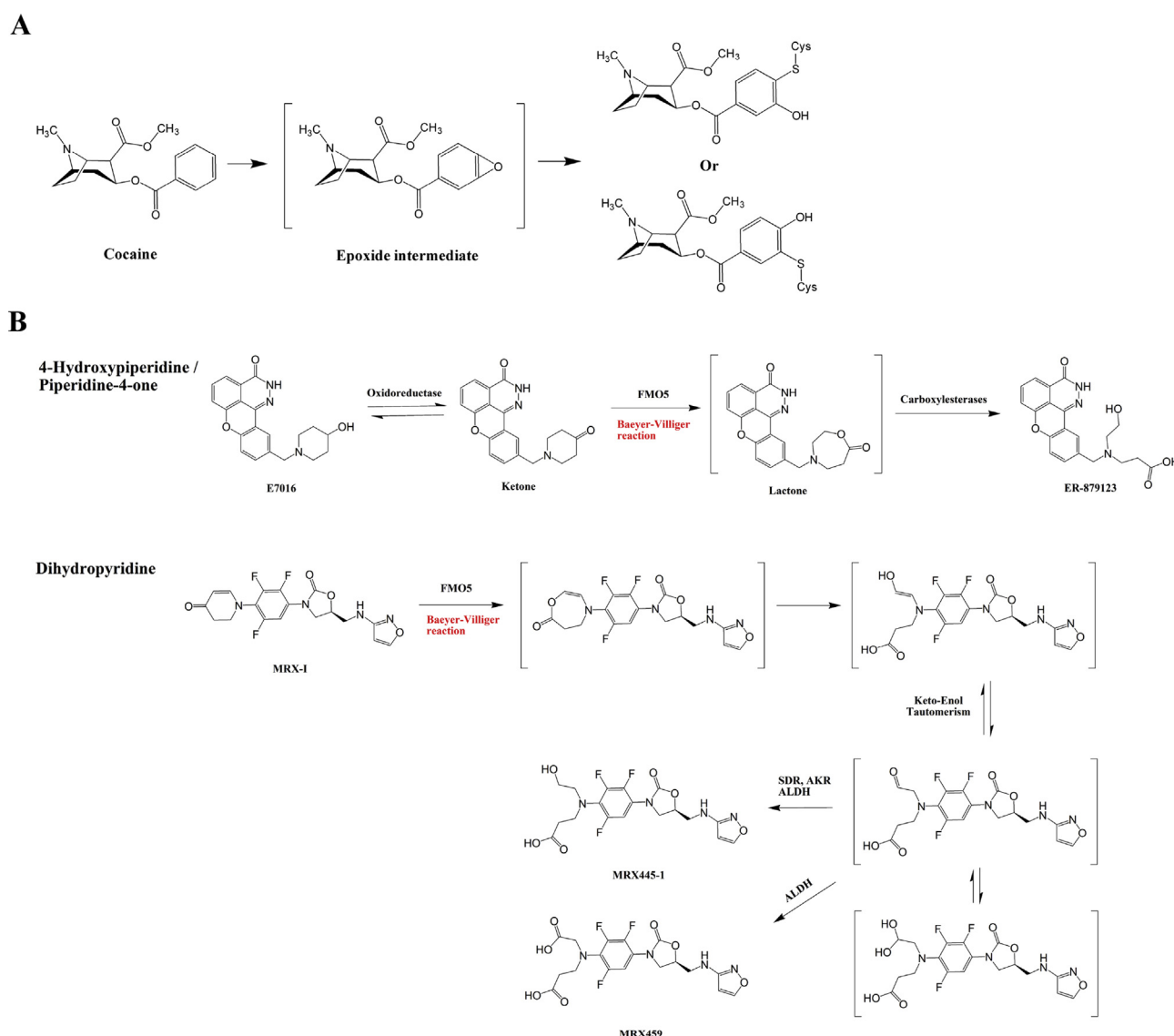
Many drugs with specific functional moieties, including esters, amides, thioesters, epoxides, sulfates, and glucuronides can be

metabolized by adding water. Hydrolysis is generally carried out by the corresponding enzymes, such as esterase or amidases. Prodrug design has received increasing interest, thereby leading to considerable attention to the important roles of hydrolytic metabolism. Some novel hydrolytic enzymes also catalyze undesirable reactions.

For example, arylacetamide deacetylase (AADAC) is a serine hydrolase expressed in human liver and intestine that is rarely reported compared with other hydrolytic enzymes (CEs and paraxonase). Only one AADAC isoform is present in humans. AADAC is identified as a lipase that is capable of hydrolyzing endogenous cholesterol ester<sup>326</sup>; however, it has been recently found to be responsible for some clinical drugs, such as prasugrel<sup>327</sup> and vicagrel<sup>328</sup>. Different from clopidogrel, the thiolactone metabolite of vicagrel is formed *via* a rapid hydrolysis before intestinal absorption<sup>329</sup>. The first activation step for vicagrel was initially believed to be mediated only by human intestine CES-2 (CES2) until a recent finding showed that AADAC also contributed to vicagrel hydrolysis (Fig. 4B). The activation of the parent

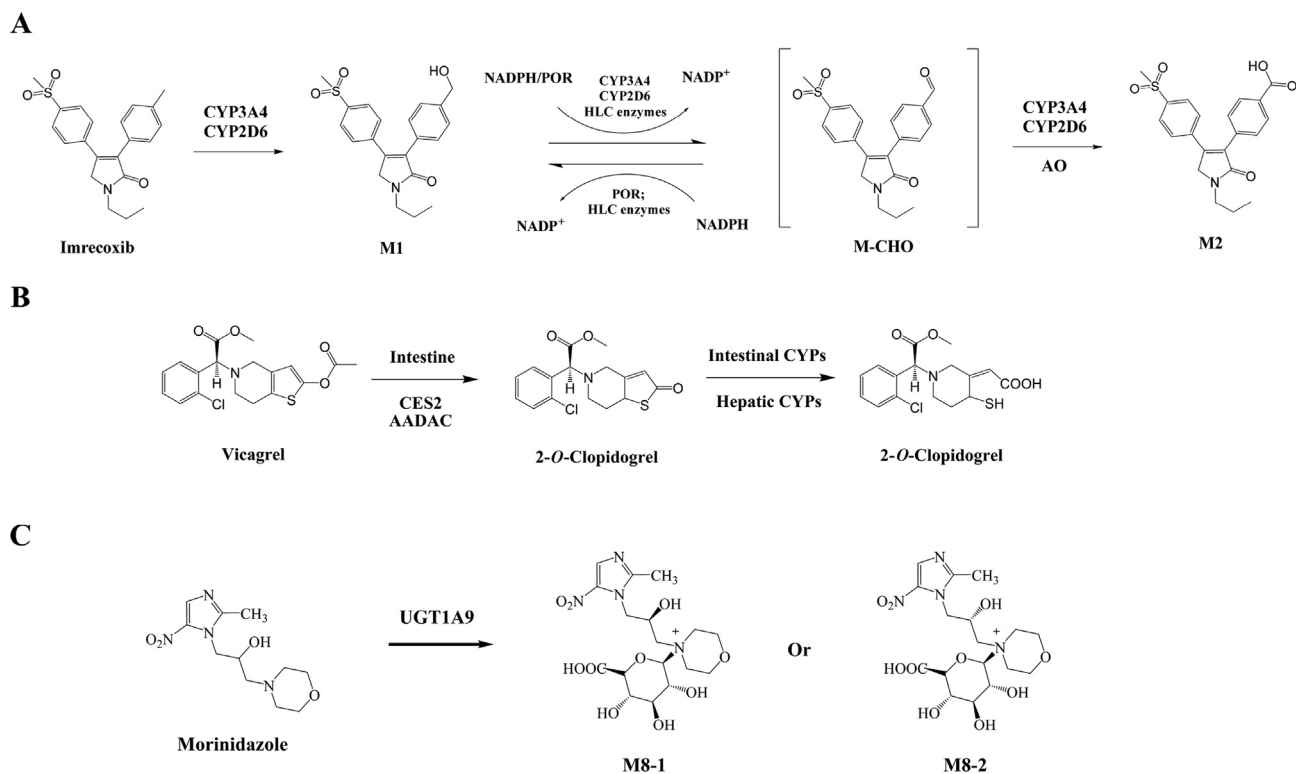
drug before entering the systemic circulation guarantees short onset time and avoidance of “clopidogrel resistance” attributed to CYP2C9 gene polymorphisms.

Another case of hydrolytic enzymes newly identified is dipeptidyl peptidases (DPPs), which can catalyze the hydrolysis of cyanopyrrolidine DDP-4 inhibitors. Generally, a nitrile group in the drug structures prevents metabolism because of its well-known inertness, and as a result, a nitrile moiety is increasing introduced as a block on metabolically labile sites in drug design<sup>330</sup>. However, for vildagliptin, anagliptin, and besigliptin (not saxagliptin), the biotransformation of the nitrile group into carboxylic acid is the major metabolic pathway *in vivo* by the DPP family such as DPP-4, DPP-2, DPP-8, DPP-9, and fibroblast activation protein- $\alpha$ <sup>331</sup>. Among them, DPP-2 has the highest hydrolytic capacity after DPP-4. However, other substrates containing a nitrile group, such as lacosamide and flutamide, cannot be hydrolyzed by DPPs probably because the nitrile moiety in these structures cannot be positioned in the catalytic triad of Asp-His-Ser of DPPs.



**Figure 3** Cases of some unusual metabolic pathways of oxidation, including: (A) proposed mechanism for cocaine metabolism to thiol-related adduction, and (B) Baeyer–Villiger oxidation mediated by FMO5.





**Figure 4** Instances of some unusual metabolic pathways for reduction, hydrolysis, and conjugation, including: (A) formation mechanism for M1 and M2, the major imrecoxib metabolites in humans, (B) hydrolysis pathways of vicagrel in humans, and (C)  $N^+$ -glucuronidation of morinidazole.

#### 7.4. Conjugation pathways

Generally, conjugation pathways involve the addition of an endogenous hydrophilic group to a drug or its metabolite(s), including glucuronidation, sulfation, glutathione conjugation, amino acid conjugation, acetylation, and methylation. Conjugation generally introduces polar groups to facilitate drug excretion, except for methylation and acetylation. Although this finding is true for many cases, several unusual conjugative reactions were reported in recent years.

The substrates for glucuronidation generally have an OH (*i.e.*, alcohols, phenol, and carboxylic acids), amino (*i.e.*, aliphatic tertiary amine, aromatic primary amine, and sulfonamide) or thiophenol group. In general, for drugs possessing both tertiary amine and hydroxyl groups, *O*-glucuronidation is always formed preferentially over *N*-glucuronidation. However, a reversible regioselectivity is observed in the conjugative metabolism of morinidazole in humans, where glucuronidation prefers the tertiary nitrogen of the morpholine ring to the aliphatic hydroxyl group at the side chain of morinidazole (Fig. 4C)<sup>332</sup>. Additionally, molecular modeling studies indicated that the regioselectivity for morinidazole glucuronidation is unrelated to steric hindrance. UGT1A4, as well as UGT1A3 and 2B10, are often considered enzymes that play important roles in  $N^+$ -glucuronidation<sup>333–335</sup>. However, according to the morinidazole experience, UGT1A9 can be identified as a new UGT isoform specializing in tertiary aliphatic amine *N*-glucuronidation.

In addition, several novel conjugates, including carnitine conjugation to cyclopropanecarboxylic acid<sup>336</sup> creatinine

conjugation to andrographolide<sup>337</sup>, and phosphoethanolamine conjugation to pimasertib<sup>338</sup>, recently have been discovered. The combination or further modification of the common conjugation process has been also reported<sup>339,340</sup>.

#### 7.5. Summary

In recent years there has been an increased effort to better understand the role of enzymes beyond P450, UDP-glucuronosyltransferase, and aldehyde oxidase in drug metabolism. Recently, several biological enzymes responsible for endogenous substrate catalysis, such as dipeptidyl peptidases and arylacetamide deacetylase, are newly proven to have additional capabilities in drug transformation. Drugs that rely on these non-P450 enzymes for their *in vivo* clearance, however, usually undergo non-classical metabolic pathways. The basic mechanism and rules of drug metabolism cannot be characterized based on the structures of the drugs alone, because the presence of metabolic intermediates that would allow for the intra-molecular rearrangement are likely factors in unusual metabolite formation. This subtle but potentially significant hypothesis suggests that the electron or radical-mediated modulation of biotransformation characteristics may represent uncommon underlying mechanisms for undesirable metabolic pathways, with relevant toxicological consequences.

Several novel and unusual reactions and pathways have been reviewed. Most of these reactions are attributed to (I) metabolic intermediate formation and rearrangement and (II) the involvement of novel metabolic enzymes, especially non-P450s.



Considering the availability of sophisticated and sensitive analytical instrumentation, as well as the introduction of modern approaches in drug metabolism investigation, new metabolic reactions continue to be discovered. Accurately predicting drug metabolism in an empirical manner and clarifying the metabolism mechanisms responsible for drug adverse reactions and drug–drug interactions will increase in the future. Additionally, valuable inspiration may be provided for rational drug design and modification with the expansion of metabolic enzymes, many of which are recognized as new therapeutic targets.

## 8. Conclusions and perspectives

DMPK research is essential for understanding the efficacy and safety of medications. Integrated studies on drug-metabolizing enzymes and transporters underlying the ADME processes as well as their transcriptional and posttranscriptional regulation mechanisms provide a comprehensive understanding of interindividual variations in pharmacotherapy. Future studies in these areas will undoubtedly advance our understanding to achieve better prediction of PK properties. Understanding the DDIs and disease–drug interactions is clinically important as such interactions may increase the risk of adverse reactions or lead to treatment failure. Although DDIs between small molecule drugs are relatively well-characterized, other potential interactions are not fully explored, including interactions with herbal biologics and other new forms of therapeutics. Furthermore, more attention should be paid to the microbiota-mediated drug interactions when examining potential DDIs and HDIs. There is emerging evidence indicating that disease–drug interactions can have a profound impact on the therapeutic outcomes. Further studies are needed to reveal the critical mechanisms by which disease–drug interactions are produced. While the benefits of PBPK are obvious for clinical trials, it is better to integrate PBPK with other modeling methods and consult experimental findings to design clinical trials in support of new drug development. Novel animal models such as those created through CRISPR-Cas9-based gene editing techniques should be an invaluable addition to current tools for PK studies. With the application of sensitive and accurate analytical instruments and technologies, many new metabolic reactions and biotransformation pathways have been and will be discovered. Predicting drug metabolism more accurately and clarifying the metabolic mechanisms responsible for adverse drug reactions and DDIs will become possible in the future. Collectively, DMPK research awaits further innovation and mechanistic studies while DMPK remains a critical component in drug development, and is essential for practicing precision medication.

## Acknowledgments

This work was supported by National Natural Science Foundation of China (grants: 81573489, 81522047, 81730103, 81320108027, 81660618, and 81773808), the National Key Research and Development Program (grant: 2017YFE0109900 and 2017YFC0909303, China), the 111 project (grant: B16047, China), the Key Laboratory Foundation of Guangdong Province (grant: 2017B030314030, China), Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT01Y093, China), National Engineering and Technology Research Center for New drug Druggability Evaluation (Seed Program of Guangdong Province, 2017B090903004, China),

Natural Science Foundation of Guangdong (grant: 2017A030311018 and 2015A030313124, China), and National Institutes of Health (grants No. R01CA225958 and R01GM113888 to Ai-Ming Yu, USA).

## Appendix A. Supporting information

Supporting data to this article can be found online at <https://doi.org/10.1016/j.apsb.2019.10.001>.

## References

- Currie GM. Pharmacology, part 2: introduction to pharmacokinetics. *J Nucl Med Technol* 2018;**46**:221–30.
- Yan R, Yang Y, Chen Y. Pharmacokinetics of Chinese medicines: strategies and perspectives. *Chin Med* 2018;**13**:24.
- Gan J, Ma S, Zhang D. Non-cytochrome P450-mediated bioactivation and its toxicological relevance. *Drug Metab Rev* 2016;**48**:473–501.
- Bhattacharyya S, Sinha K, Sil PC. Cytochrome P450s: mechanisms and biological implications in drug metabolism and its interaction with oxidative stress. *Curr Drug Metab* 2014;**15**:719–42.
- Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer* 2006;**6**:947–60.
- Yu Z, Tian X, Peng Y, Sun Z, Wang C, Tang N, et al. Mitochondrial cytochrome P450 (CYP) 1B1 is responsible for melatonin-induced apoptosis in neural cancer cells. *J Pineal Res* 2018;**65**:e12478.
- Ruwali M, Dhawan A, Pant MC, Rahman Q, Khurana SM, Parmar D. Clinical management of head and neck cancer cases: role of pharmacogenetics of CYP2 and GSTs. *Oncol Res Treat* 2016;**39**:221–6.
- Wang X, Li J, Dong G, Yue J. The endogenous substrates of brain CYP2D. *Eur J Pharmacol* 2014;**724**:211–8.
- Yi M, Cho SA, Min J, Kim DH, Shin JG, Lee SJ. Functional characterization of a common CYP4F11 genetic variant and identification of functionally defective CYP4F11 variants in erythromycin metabolism and 20-HETE synthesis. *Arch Biochem Biophys* 2017;**620**:43–51.
- Deng J, Guo L, Wu B. Circadian regulation of hepatic cytochrome P450 2a5 by peroxisome proliferator-activated receptor  $\gamma$ . *Drug Metab Dispos* 2018;**46**:1538–45.
- Cannady EA, Suico JG, Wang MD, Friedrich S, Rehmel JR, Nicholls SJ, et al. CYP-mediated drug–drug interactions with evacetrapi, an investigational CETP inhibitor: *in vitro* prediction and clinical outcome. *Br J Clin Pharmacol* 2015;**80**:1388–98.
- Siu EC, Tyndale RF. Selegiline is a mechanism-based inactivator of CYP2A6 inhibiting nicotine metabolism in humans and mice. *J Pharmacol Exp Ther* 2008;**324**:992–9.
- Pelkonen O, Rautio A, Raunio H, Pasanen M. CYP2A6: a human coumarin 7-hydroxylase. *Toxicology* 2000;**144**:139–47.
- He XY, Shen J, Ding X, Lu AY, Hong JY. Identification of critical amino acid residues of human CYP2A13 for the metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-specific carcinogen. *Drug Metab Dispos* 2004;**32**:1516–21.
- Li L, Carratt S, Hartog M, Kovalchuk N, Jia K, Wang Y, et al. Human CYP2A13 and CYP2F1 mediate naphthalene toxicity in the lung and nasal mucosa of CYP2A13/2F1-humanized mice. *Environ Health Perspect* 2017;**125**:067004.
- Xu M, Hao H, Jiang L, Long F, Wei Y, Ji H, et al. *In vitro* inhibitory effects of ethanol extract of Danshen (*Salvia miltiorrhiza*) and its components on the catalytic activity of soluble epoxide hydrolase. *Phytomedicine* 2015;**22**:444–51.
- Xie C, Gao X, Sun D, Zhang Y, Krausz KW, Qin X, et al. Metabolic profiling of the novel hypoxia-inducible factor 2 $\alpha$  inhibitor PT2385 *in vivo* and *in vitro*. *Drug Metab Dispos* 2018;**46**:336–45.

18. Zhao H, Li S, Yang Z, Peng Y, Chen X, Zheng J. Identification of ketene-reactive intermediate of erlotinib possibly responsible for inactivation of P450 enzymes. *Drug Metab Dispos* 2018;**46**:442–50.
19. Liu L, Cao X, Li T, Li X. Effects of catalpol on the activity of human liver cytochrome P450 enzymes. *Xenobiotica* 2019;**49**:1289–95.
20. Yim D, Kim MJ, Shin Y, Lee SJ, Shin JG, Kim DH. Inhibition of cytochrome P450 activities by *Sophora flavescens* extract and its prenylated flavonoids in human liver microsomes. *Evid Based Complement Alternat Med* 2019;**2019**:2673769.
21. Chen P, Li D, Chen Y, Sun J, Fu K, Guan L, et al. p53-mediated regulation of bile acid disposition attenuates cholic acid-induced cholestasis in mice. *Br J Pharmacol* 2017;**174**:4345–61.
22. Showande SJ, Fakeye TO, Kajula M, Hokkanen J, Tolonen A. Potential inhibition of major human cytochrome P450 isoenzymes by selected tropical medicinal herbs-Implication for herb–drug interactions. *Food Sci Nutr* 2019;**7**:44–55.
23. Alnaqeeb M, Mansor KA, Mallah EM, Ghanim BY, Idkaidek N, Qinna NA. Critical pharmacokinetic and pharmacodynamic drug-herb interactions in rats between warfarin and pomegranate peel or guava leaves extracts. *BMC Complement Altern Med* 2019;**19**:29.
24. Yang J, He MM, Niu W, Wrigton SA, Li L, Liu Y, et al. Metabolic capabilities of cytochrome P450 enzymes in Chinese liver microsomes compared with those in Caucasian liver microsomes. *Br J Clin Pharmacol* 2012;**73**:268–84.
25. Gao N, Tian X, Fang Y, Zhou J, Zhang H, Wen Q, et al. Gene polymorphisms and contents of cytochrome P450s have only limited effects on metabolic activities in human liver microsomes. *Eur J Pharm Sci* 2016;**92**:86–97.
26. Li GF, Zheng QS, Yu Y, Zhong W, Zhou HH, Qiu F, et al. Impact of ethnicity-specific hepatic microsomal scaling factor, liver weight, and cytochrome P450 (CYP) 1A2 content on physiologically based prediction of CYP1A2-mediated pharmacokinetics in young and elderly Chinese adults. *Clin Pharmacokinet* 2019;**58**:927–41.
27. Kaminsky LS, Zhang QY. The small intestine as a xenobiotic-metabolizing organ. *Drug Metab Dispos* 2003;**31**:1520–5.
28. Knights KM, Rowland A, Miners JQ. Renal drug metabolism in humans: the potential for drug-endobiotic interactions involving cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT). *Br J Clin Pharmacol* 2013;**76**:587–602.
29. Edeogu CO, Kalu ME, Famurewa AC, Asogwa NT, Onyeji GN, Ikpeko KO. Nephroprotective effect of *Moringa oleifera* seed oil on gentamicin-induced nephrotoxicity in rats: biochemical evaluation of antioxidant, anti-inflammatory, and antiapoptotic pathways. *J Am Coll Nutr* 2019;**12**:1–9.
30. Liu H, Chen M, Yin H, Hu P, Wang Y, Liu F, et al. Exploration of the hepatoprotective chemical base of an orally administered herbal formulation (YCHT) in normal and CCl<sub>4</sub>-intoxicated liver injury rats. Part 1: metabolic profiles from the liver-centric perspective. *J Ethnopharmacol* 2019;**237**:81–91.
31. Marchitti SA, Brocker C, Stagos D, Vasilou V. Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. *Expert Opin Drug Metab Toxicol* 2008;**4**:697–720.
32. Miyajima A, Sakemi-Hoshikawa K, Usami M, Mitsunaga K, Irie T, Ohno Y, et al. Thyrotoxic rubber antioxidants, 2-mercaptobenzimidazole and its methyl derivatives, cause both inhibition and induction of drug-metabolizing activity in rat liver microsomes after repeated oral administration. *Biochem Biophys Res Commun* 2017;**492**:116–20.
33. Di Paolo ML, Cozza G, Milelli A, Zonta F, Sarno S, Minniti E, et al. Benextramine and derivatives as novel human monoamine oxidases inhibitors: an integrated approach. *FEBS J* 2019. Available from: <https://doi.org/10.1111/febs.14994>.
34. Tao D, Wang Y, Bao XQ, Yang BB, Gao F, Wang L, et al. Discovery of coumarin Mannich base derivatives as multifunctional agents against monoamine oxidase B and neuroinflammation for the treatment of Parkinson's disease. *Eur J Med Chem* 2019;**173**:203–12.
35. Foti RS, Dalvie DK. Cytochrome P450 and non-cytochrome P450 oxidative metabolism: contributions to the pharmacokinetics, safety, and efficacy of xenobiotics. *Drug Metab Dispos* 2016;**44**:1229–45.
36. Johnson P, Loganathan C, Iruthayaraj A, Poomani K, Thayumanavan P. S-allyl cysteine as potent anti-gout drug: insight into the xanthine oxidase inhibition and anti-inflammatory activity. *Biochimie* 2018;**154**:1–9.
37. Zhang HF, Li ZH, Liu JY, Liu TT, Wang P, Fang Y, et al. Correlation of cytochrome P450 oxidoreductase expression with the expression of 10 isoforms of cytochrome P450 in human liver. *Drug Metab Dispos* 2016;**44**:1193–200.
38. Mano EC, Scott AL, Honorio KM. UDP-glucuronosyltransferases: structure, function and drug design studies. *Curr Med Chem* 2018;**25**:3247–55.
39. Mazerska Z, Mróz A, Pawlowska M, Augustin E. The role of glucuronidation in drug resistance. *Pharmacol Ther* 2016;**159**:35–55.
40. Nair PC, Meech R, Mackenzie PI, McKinnon RA, Miners JO. Insights into the UDP-sugar selectivities of human UDP-glycosyltransferases (UGT): a molecular modeling perspective. *Drug Metab Rev* 2015;**47**:335–45.
41. Qi C, Fu J, Zhao H, Xing H, Dong D, Wu B. Identification of UGTs and BCRP as potential pharmacokinetic determinants of the natural flavonoid alpinetin. *Xenobiotica* 2019;**49**:276–83.
42. Pettersson Bergstrand M, Richter LH, Maurer HH, Wagmann L, Meyer MR. *In vitro* glucuronidation of designer benzodiazepines by human UDP-glucuronosyltransferases. *Drug Test Anal* 2019;**11**:45–50.
43. Hu DG, Meech R, McKinnon RA, Mackenzie PI. Transcriptional regulation of human UDP-glucuronosyltransferase genes. *Drug Metab Rev* 2014;**46**:421–58.
44. Gao R, Liu M, Chen Y, Xia C, Zhang H, Xiong Y, et al. Identification and characterization of human UDP-glucuronosyltransferases responsible for the *in vitro* glucuronidation of ursolic acid. *Drug Metab Pharmacokinet* 2016;**31**:261–8.
45. Jeong ES, Kim YW, Kim HJ, Shin HJ, Shin JG, Kim KH, et al. Glucuronidation of fimasartan, a new angiotensin receptor antagonist, is mainly mediated by UGT1A3. *Xenobiotica* 2015;**45**:10–8.
46. Wang H, Cao G, Wang G, Hao H. Regulation of mammalian UDP-glucuronosyltransferases. *Curr Drug Metab* 2018;**19**:490–501.
47. Zhou X, Zhao Y, Wang J, Wang X, Chen C, Yin D, et al. Resveratrol represses estrogen-induced mammary carcinogenesis through NRF2-UGT1A8-estrogen metabolic axis activation. *Biochem Pharmacol* 2018;**155**:252–63.
48. Wu L, Chen Y, Liu H, Zhan Z, Liang Z, Zhang T, et al. Emodin-induced hepatotoxicity was exacerbated by probenecid through inhibiting UGTs and MRP2. *Toxicol Appl Pharmacol* 2018;**359**:91–101.
49. Zhou Y, Cao S, Wang Y, Xu P, Yan J, Bin W, et al. Berberine metabolites could induce low density lipoprotein receptor up-regulation to exert lipid-lowering effects in human hepatoma cells. *Fitoterapia* 2014;**92**:230–7.
50. Hwang DK, Kim JH, Shin Y, Choi WG, Kim S, Cho YY, et al. Identification of catalposide metabolites in human liver and intestinal preparations and characterization of the relevant sulfotransferase, UDP-glucuronosyltransferase, and carboxylesterase enzymes. *Pharmaceutics* 2019;**11**:355.
51. Wassenaar CA, Conti DV, Das S, Chen P, Cook EH, Ratain MJ, et al. *UGT1A* and *UGT2B* genetic variation alters nicotine and nitrosamine glucuronidation in european and african american smokers. *Cancer Epidemiol Biomarkers Prev* 2015;**24**:94–104.
52. Suh HJ, Yoon SH, Yu KS, Cho JY, Park SI, Lee E, et al. The genetic polymorphism *UGT1A4*\*3 is associated with low posaconazole plasma concentrations in hematological malignancy patients receiving the oral suspension. *Antimicrob Agents Chemother* 2018;**62**:e02230-17.
53. Zhang X, Yin JF, Zhang J, Kong SJ, Zhang HY, Chen XM. *UGT1A1*\*6 polymorphisms are correlated with irinotecan-induced neutropenia: a systematic review and meta-analysis. *Cancer Chemother Pharmacol* 2017;**80**:135–49.

54. Suiko M, Kurogi K, Hashiguchi T, Sakakibara Y, Liu MC. Updated perspectives on the cytosolic sulfotransferases (SULTs) and SULT-mediated sulfation. *Biosci Biotechnol Biochem* 2016;**81**:63–72.
55. Wang S, Yuan X, Lu D, Guo L, Wu B. Farnesoid X receptor regulates SULT1E1 expression through inhibition of PGC1 $\alpha$  binding to HNF4 $\alpha$ . *Biochem Pharmacol* 2017;**145**:202–9.
56. Falany CN, Rohn-Glowacki KJ. SULT2B1: unique properties and characteristics of a hydroxysteroid sulfotransferase family. *Drug Metab Rev* 2013;**45**:388–400.
57. Sun J, Wen Y, Zhou Y, Jiang Y, Chen Y, Zhang H, et al. p53 attenuates acetaminophen-induced hepatotoxicity by regulating drug-metabolizing enzymes and transporter expression. *Cell Death Dis* 2018;**9**:536.
58. Li R, Liu F, Chang Y, Ma X, Li M, Li C, et al. Glutathione S-transferase A1 (GSTA1) as a marker of acetaminophen-induced hepatocyte injury *in vitro*. *Toxicol Mech Methods* 2017;**27**:401–7.
59. Yang Y, Huycke MM, Herman TS, Wang X. Glutathione S-transferase alpha 4 induction by activator protein 1 in colorectal cancer. *Oncogene* 2016;**35**:5795–806.
60. Yi M, Shin JG, Lee SJ. Expression of CYP4V2 in human THP1 macrophages and its transcriptional regulation by peroxisome proliferator-activated receptor gamma. *Toxicol Appl Pharmacol* 2017;**330**:100–6.
61. Jeong SJ, Park JG, Kim S, Kweon HY, Seo S, Na DS, et al. Extract of *Rhus verniciflua* stokes protects the diet-induced hyperlipidemia in mice. *Arch Pharm Res* 2015;**38**:2049–58.
62. Graham A. Mitochondrial regulation of macrophage cholesterol homeostasis. *Free Radic Biol Med* 2015;**89**:982–92.
63. Saini SP, Zhang B, Niu Y, Jiang M, Gao J, Zhai Y, et al. Activation of liver X receptor increases acetaminophen clearance and prevents its toxicity in mice. *Hepatology* 2011;**54**:2208–17.
64. Kalthoff S, Winkler A, Freiberg N, Manns MP, Strassburg CP. Gender matters: estrogen receptor alpha (ER $\alpha$ ) and histone deacetylase (HDAC) 1 and 2 control the gender-specific transcriptional regulation of human uridine diphosphate glucuronosyltransferases genes (UGT1A). *J Hepatol* 2013;**59**:797–804.
65. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* 2001;**11**:1156–66.
66. Shugarts S, Benet LZ. The role of transporters in the pharmacokinetics of orally administered drugs. *Pharm Res* 2009;**26**:2039–54.
67. Kota BP, Tran VH, Allen J, Bebawy M, Roufogalis BD. Characterization of PXR mediated P-glycoprotein regulation in intestinal LS174T cells. *Pharmacol Res* 2010;**62**:426–31.
68. Lopes-Rodrigues V, Seca H, Sousa D, Sousa E, Lima RT, Vasconcelos MH. The network of P-glycoprotein and microRNAs interactions. *Int J Cancer* 2014;**135**:253–63.
69. Sun Y, Wang C, Meng Q, Liu Z, Huo X, Sun P, et al. Targeting P-glycoprotein and SORCIN: dihydromyricetin strengthens anti-proliferative efficiency of adriamycin *via* MAPK/ERK and Ca<sup>2+</sup>-mediated apoptosis pathways in MCF-7/ADR and K562/ADR. *J Cell Physiol* 2018;**233**:3066–79.
70. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer* 2018;**18**:452–64.
71. Rives ML, Javitch JA, Wickenden AD. Potentiating SLC transporter activity: emerging drug discovery opportunities. *Biochem Pharmacol* 2017;**135**:1–11.
72. Rocha KC, Pereira BM, Rodrigues AC. An update on efflux and uptake transporters as determinants of statin response. *Expert Opin Drug Metab Toxicol* 2018;**14**:613–24.
73. Zakeri-Milani P, Valizadeh H. Intestinal transporters: enhanced absorption through P-glycoprotein-related drug interactions. *Expert Opin Drug Metab Toxicol* 2014;**10**:859–71.
74. Yu J, Zhou Z, Tay-Sontheimer J, Levy RH, Ragueneau-Majlessi I. Intestinal drug interactions mediated by OATPs: a systematic review of preclinical and clinical findings. *J Pharm Sci* 2017;**106**:2312–25.
75. Oostendorp RL, Beijnen JH, Schellens JH. The biological and clinical role of drug transporters at the intestinal barrier. *Cancer Treat Rev* 2009;**35**:137–47.
76. Cheng Z, VanPelt J, Bergstrom A, Bethel C, Katko A, Miller C, et al. A noncanonical metal center drives the activity of the *Sediminispirochaeta smaragdinae* metallo- $\beta$ -lactamase SPS-1. *Biochemistry* 2018;**57**:5218–29.
77. Zhang J, Wang C, Liu Q, Meng Q, Cang J, Sun H, et al. Pharmacokinetic interaction between JBP485 and cephalexin in rats. *Drug Metab Dispos* 2010;**38**:930–8.
78. Cang J, Zhang J, Wang C, Liu Q, Meng Q, Wang D, et al. Pharmacokinetics and mechanism of intestinal absorption of JBP485 in rats. *Drug Metab Pharmacokinet* 2010;**25**:500–7.
79. Nieto Montesinos R, Béduneau A, Pellequer Y, Lamprecht A. Delivery of P-glycoprotein substrates using chemosensitizers and nanotechnology for selective and efficient therapeutic outcomes. *J Control Release* 2012;**161**:50–61.
80. Pedersen KE, Christiansen BD, Klitgaard NA, Nielsen-Kudsk F. Effect of quinidine on digoxin bioavailability. *Eur J Clin Pharmacol* 1983;**24**:41–7.
81. Huo X, Liu Q, Wang C, Meng Q, Sun H, Peng J, et al. Enhancement effect of P-gp inhibitors on the intestinal absorption and anti-proliferative activity of bestatin. *Eur J Pharm Sci* 2013;**50**:420–8.
82. Yigitaslan S, Erol K, Cengelli C. The effect of P-glycoprotein inhibition and activation on the absorption and serum levels of cyclosporine and tacrolimus in rats. *Adv Clin Exp Med* 2016;**25**:237–42.
83. Karibe T, Imaoka T, Abe K, Ando O. Curcumin as an *in vivo* selective intestinal breast cancer resistance protein inhibitor in cynomolgus monkeys. *Drug Metab Dispos* 2018;**46**:667–79.
84. Yamagata T, Kusuhara H, Morishita M, Takayama K, Benameur H, Sugiyama Y. Effect of excipients on breast cancer resistance protein substrate uptake activity. *J Control Release* 2007;**124**:1–5.
85. Jia Y, Liu Z, Wang C, Meng Q, Huo X, Liu Q, et al. P-gp, MRP2 and OAT1/OAT3 mediate the drug–drug interaction between resveratrol and methotrexate. *Toxicol Appl Pharmacol* 2016;**306**:27–35.
86. Wang C, Huo X, Wang C, Meng Q, Liu Z, Sun P, et al. Organic anion-transporting polypeptide and efflux transporter-mediated hepatic uptake and biliary excretion of cilostazol and its metabolites in Rats and humans. *J Pharm Sci* 2017;**106**:2515–23.
87. Watanabe T, Kusuhara H, Maeda K, Shitara Y, Sugiyama Y. Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. *J Pharmacol Exp Ther* 2009;**328**:652–62.
88. De Lange EC, Vd Berg DJ, Bellanti F, Voskuyl R, Syvänen S. P-glycoprotein protein expression *versus* functionality at the blood–brain barrier using immunohistochemistry, microdialysis and mathematical modeling. *Eur J Pharm Sci* 2018;**124**:61–70.
89. Hartz AM, Zhong Y, Shen AN, Abner EL, Bauer B. Preventing P-gp ubiquitination lowers A $\beta$  brain levels in an Alzheimer's disease mouse model. *Front Aging Neurosci* 2018;**10**:186.
90. Ohtsuki S, Asaba H, Takanaga H, Deguchi T, Hosoya KI, Otagiri M, et al. Role of blood–brain barrier organic anion transporter 3 (OAT3) in the efflux of indoxyl sulfate, a uremic toxin: its involvement in neurotransmitter metabolite clearance from the brain. *J Neurochem* 2002;**83**:57–66.
91. Matsson EM, Eriksson UG, Palm JE, Artursson P, Karlgren M, Lazorova L, et al. Combined *in vitro*–*in vivo* approach to assess the hepatobiliary disposition of a novel oral thrombin inhibitor. *Mol Pharm* 2013;**10**:4252–62.
92. Notenboom S, Weigand KM, Proost JH, van Lipzig MM, van de Steeg E, van den Broek PH, et al. Development of a mechanistic biokinetic model for hepatic bile acid handling to predict possible cholestatic effects of drugs. *Eur J Pharm Sci* 2018;**115**:175–84.
93. Zhu Y, Meng Q, Wang C, Liu Q, Sun H, Kaku T, et al. Organic anion transporters involved in the excretion of bestatin in the kidney. *Peptides* 2012;**33**:265–71.



94. Guo X, Meng Q, Liu Q, Wang C, Mao Q, Sun H, et al. Peptide cotransporter 1 in intestine and organic anion transporters in kidney are targets of interaction between JBP485 and lisinopril in rats. *Drug Metab Pharmacokinet* 2012;**27**:232–41.
95. Ye J, Liu Q, Wang C, Meng Q, Peng J, Sun H, et al. Inhibitory effect of JBP485 on renal excretion of acyclovir by the inhibition of OAT1 and OAT3. *Eur J Pharm Sci* 2012;**47**:341–6.
96. Xu Q, Wang C, Meng Q, Liu Q, Sun H, Peng J, et al. OAT1 and OAT3: targets of drug–drug interaction between entecavir and JBP485. *Eur J Pharm Sci* 2013;**48**:650–7.
97. Takeda M, Tojo A, Sekine T, Hosoyamada M, Kanai Y, Endou H. Role of organic anion transporter 1 (OAT1) in cephaloridine (CER)-induced nephrotoxicity. *Kidney Int* 1999;**56**:2128–36.
98. Jung KY, Takeda M, Shimoda M, Narikawa S, Tojo A, Kim DK, et al. Involvement of rat organic anion transporter 3 (rOAT3) in cephaloridine-induced nephrotoxicity: in comparison with rOAT1. *Life Sci* 2002;**70**:1861–74.
99. Müller F, Weitz D, Mertsch K, König J, Fromm MF. Importance of OCT2 and MATE1 for the cimetidine-metformin interaction: insights from investigations of polarized transport in single- and double-transfected MDCK cells with a focus on perpetrator disposition. *Mol Pharm* 2018;**15**:3425–33.
100. El-Arabey AA. Dual function of OCT2 and MATE1 in cisplatin induced nephrotoxicity. *Pharmacol Res* 2017;**119**:493.
101. Zhu Y, Meng Q, Wang C, Liu Q, Huo X, Zhang A, et al. Methotrexate-bestatin interaction: involvement of P-glycoprotein and organic anion transporters in rats. *Int J Pharm* 2014;**465**:368–77.
102. Zhang R, Yang X, Li J, Wu J, Peng WX, Dong XQ, et al. Upregulation of rat renal cortical organic anion transporter (OAT1 and OAT3) expression in response to ischemia/reperfusion injury. *Am J Nephrol* 2008;**28**:772–83.
103. Mattick JS. RNA regulation: a new genetics?. *Nat Rev Genet* 2004;**5**:316–23.
104. Yu AM, Tian Y, Tu MJ, Ho PY, Jilek JL. MicroRNA pharmacoeugenetics: posttranscriptional regulation mechanisms behind variable drug disposition and strategy to develop more effective therapy. *Drug Metab Dispos* 2016;**44**:308–19.
105. Yu AM, Ingelman-Sundberg M, Cherrington NJ, Aleksunes LM, Zanger UM, Xie W, et al. Regulation of drug metabolism and toxicity by multiple factors of genetics, epigenetics, lncRNAs, gut microbiota, and diseases: a meeting report of the 21st International Symposium on Microsomes and Drug Oxidations (MDO). *Acta Pharm Sin B* 2017;**7**:241–8.
106. Nakano M, Nakajima M. Current knowledge of microRNA-mediated regulation of drug metabolism in humans. *Expert Opin Drug Metab Toxicol* 2018;**14**:493–504.
107. Tsuchiya Y, Nakajima M, Takagi S, Taniya T, Yokoi T. MicroRNA regulates the expression of human cytochrome P450 1B1. *Cancer Res* 2006;**66**:9090–8.
108. Pan YZ, Gao W, Yu AM. MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. *Drug Metab Dispos* 2009;**37**:2112–7.
109. Li MM, Wang WP, Wu WJ, Huang M, Yu AM. Rapid production of novel pre-microRNA agent hsa-mir-27b in *Escherichia coli* using recombinant RNA technology for functional studies in mammalian cells. *Drug Metab Dispos* 2014;**42**:1791–5.
110. Li X, Tian Y, Tu MJ, Ho PY, Batra N, Yu AM. Bioengineered miR-27b-3p and miR-328-3p modulate drug metabolism and disposition via the regulation of target ADME gene expression. *Acta Pharm Sin B* 2019;**9**:639–47.
111. Zhu H, Wu H, Liu X, Evans BR, Medina DJ, Liu CG, et al. Role of microRNA miR-27a and miR-451 in the regulation of *MDR1/P-glycoprotein* expression in human cancer cells. *Biochem Pharmacol* 2008;**76**:582–8.
112. Li Z, Hu S, Wang J, Cai J, Xiao L, Yu L, et al. MiR-27a modulates *MDR1/P-glycoprotein* expression by targeting *HIPK2* in human ovarian cancer cells. *Gynecol Oncol* 2010;**119**:125–30.
113. Feng DD, Zhang H, Zhang P, Zheng YS, Zhang XJ, Han BW, et al. Down-regulated miR-331-5p and miR-27a are associated with chemotherapy resistance and relapse in leukaemia. *J Cell Mol Med* 2011;**15**:2164–75.
114. Papageorgiou I, Court MH. Identification and validation of the microRNA response elements in the 3'-untranslated region of the UDP glucuronosyltransferase (*UGT*) 2B7 and 2B15 genes by a functional genomics approach. *Biochem Pharmacol* 2017;**146**:199–213.
115. Papageorgiou I, Court MH. Identification and validation of microRNAs directly regulating the UDP-glucuronosyltransferase 1A subfamily enzymes by a functional genomics approach. *Biochem Pharmacol* 2017;**137**:93–106.
116. Dluzen DF, Sun D, Salzberg AC, Jones N, Bushey RT, Robertson GP, et al. Regulation of UDP-glucuronosyltransferase 1A1 expression and activity by microRNA 491-3p. *J Pharmacol Exp Ther* 2014;**348**:465–77.
117. Wijayakumara DD, Hu DG, Meech R, McKinnon RA, Mackenzie PI. Regulation of human UGT2B15 and UGT2B17 by miR-376c in prostate cancer cell lines. *J Pharmacol Exp Ther* 2015;**354**:417–25.
118. Tatsumi N, Tokumitsu S, Nakano M, Fukami T, Nakajima M. miR-141-3p commonly regulates human UGT1A isoforms via different mechanisms. *Drug Metab Pharmacokinet* 2018;**33**:203–10.
119. Sutliff AK, Watson CJ, Chen G, Lazarus P. Regulation of UGT2A1 by miR-196a-5p and miR-196b-5p. *J Pharmacol Exp Ther* 2019;**369**:234–43.
120. Yu D, Green B, Tolleson WH, Jin Y, Mei N, Guo Y, et al. MicroRNA hsa-miR-29a-3p modulates CYP2C19 in human liver cells. *Biochem Pharmacol* 2015;**98**:215–23.
121. Mencia N, Selga E, Noé V, Ciudad CJ. Underexpression of miR-224 in methotrexate resistant human colon cancer cells. *Biochem Pharmacol* 2011;**82**:1572–82.
122. Wang Y, Yu D, Tolleson WH, Yu LR, Green B, Zeng L, et al. A systematic evaluation of microRNAs in regulating human hepatic CYP2E1. *Biochem Pharmacol* 2017;**138**:174–84.
123. Chen Y, Zeng L, Wang Y, Tolleson WH, Knox B, Chen S, et al. The expression, induction and pharmacological activity of CYP1A2 are post-transcriptionally regulated by microRNA hsa-miR-132-5p. *Biochem Pharmacol* 2017;**145**:178–91.
124. Zeng L, Chen Y, Wang Y, Yu LR, Knox B, Chen J, et al. MicroRNA hsa-miR-370-3p suppresses the expression and induction of CYP2D6 by facilitating mRNA degradation. *Biochem Pharmacol* 2017;**140**:139–49.
125. Yu D, Wu L, Gill P, Tolleson WH, Chen S, Sun J, et al. Multiple microRNAs function as self-protective modules in acetaminophen-induced hepatotoxicity in humans. *Arch Toxicol* 2018;**92**:845–58.
126. Duan Z, Yu AM. Bioengineered non-coding RNA agent (BERA) in action. *Bioengineered* 2016;**7**:411–7.
127. Ho PY, Yu AM. Bioengineering of noncoding RNAs for research agents and therapeutics. *Wiley Interdiscip Rev RNA* 2016;**7**:186–97.
128. Yu AM, Jian C, Yu AH, Tu MJ. RNA therapy: are we using the right molecules?. *Pharmacol Ther* 2019;**196**:91–104.
129. Chen QX, Wang WP, Zeng S, Urayama S, Yu AM. A general approach to high-yield biosynthesis of chimeric RNAs bearing various types of functional small RNAs for broad applications. *Nucleic Acids Res* 2015;**43**:3857–69.
130. Li MM, Addepalli B, Tu MJ, Chen QX, Wang WP, Limbach PA, et al. Chimeric microRNA-1291 biosynthesized efficiently in *Escherichia coli* is effective to reduce target gene expression in human carcinoma cells and improve chemosensitivity. *Drug Metab Dispos* 2015;**43**:1129–36.
131. Ho PY, Duan Z, Batra N, Jilek JL, Tu MJ, Qiu JX, et al. Bioengineered noncoding RNAs selectively change cellular miRNome profiles for cancer therapy. *J Pharmacol Exp Ther* 2018;**365**:494–506.
132. Li PC, Tu MJ, Ho PY, Jilek JL, Duan Z, Zhang QY, et al. Bioengineered NRF2-siRNA is effective to interfere with NRF2 pathways and improve chemosensitivity of human cancer cells. *Drug Metab Dispos* 2018;**46**:2–10.

133. Wang WP, Ho PY, Chen QX, Addepalli B, Limbach PA, Li MM, et al. Bioengineering novel chimeric microRNA-34a for prodrug cancer therapy: high-yield expression and purification, and structural and functional characterization. *J Pharmacol Exp Ther* 2015;**354**:131–41.
134. Jilek JL, Tian Y, Yu AM. Effects of microRNA-34a on the pharmacokinetics of cytochrome P450 probe drugs in mice. *Drug Metab Dispos* 2017;**45**:512–22.
135. Zhao Y, Tu MJ, Yu YF, Wang WP, Chen QX, Qiu JX, et al. Combination therapy with bioengineered miR-34a prodrug and doxorubicin synergistically suppresses osteosarcoma growth. *Biochem Pharmacol* 2015;**98**:602–13.
136. Jian C, Tu MJ, Ho PY, Duan Z, Zhang Q, Qiu JX, et al. Co-targeting of DNA, RNA, and protein molecules provides optimal outcomes for treating osteosarcoma and pulmonary metastasis in spontaneous and experimental metastasis mouse models. *Oncotarget* 2017;**8**:30742–55.
137. Jilek JL, Zhang QY, Tu MJ, Ho PY, Duan Z, Qiu JX, et al. Bioengineered Let-7c inhibits orthotopic hepatocellular carcinoma and improves overall survival with minimal immunogenicity. *Mol Ther Nucleic Acids* 2019;**14**:498–508.
138. Tu MJ, Ho PY, Zhang QY, Jian C, Qiu JX, Kim EJ, et al. Bioengineered miRNA-1291 prodrug therapy in pancreatic cancer cells and patient-derived xenograft mouse models. *Cancer Lett* 2019;**442**:82–90.
139. Alegre F, Ormonde AR, Snider KM, Woolard K, Yu AM, Wittenburg LA. A genetically engineered microRNA-34a prodrug demonstrates anti-tumor activity in a canine model of osteosarcoma. *PLoS One* 2018;**13**:e0209941.
140. Chen L, Bao Y, Piekos SC, Zhu K, Zhang L, Zhong XB. A transcriptional regulatory network containing nuclear receptors and long non-coding RNAs controls basal and drug-induced expression of cytochrome P450s in hepaRG cells. *Mol Pharmacol* 2018;**94**:749–59.
141. Tsang WP, Kwok TT. Riboregulator H19 induction of MDR1-associated drug resistance in human hepatocellular carcinoma cells. *Oncogene* 2007;**26**:4877–81.
142. Wang Y, Zhang D, Wu K, Zhao Q, Nie Y, Fan D. Long noncoding RNA *MRUL* promotes *ABCB1* expression in multidrug-resistant gastric cancer cell sublines. *Mol Cell Biol* 2014;**34**:3182–93.
143. Wang J, Ye C, Liu J, Hu Y. UCA1 confers paclitaxel resistance to ovarian cancer through miR-129/ABCB1 axis. *Biochem Biophys Res Commun* 2018;**501**:1034–40.
144. Han Z, Shi L. Long non-coding RNA LUCAT1 modulates methotrexate resistance in osteosarcoma via miR-200c/ABCB1 axis. *Biochem Biophys Res Commun* 2018;**495**:947–53.
145. Nakano M, Fukami T, Gotoh S, Nakajima M. A-to-I RNA editing up-regulates human dihydrofolate reductase in breast cancer. *J Biol Chem* 2017;**292**:4873–84.
146. Nakano M, Fukami T, Gotoh S, Takamiya M, Aoki Y, Nakajima M. RNA editing modulates human hepatic aryl hydrocarbon receptor expression by creating microRNA recognition sequence. *J Biol Chem* 2016;**291**:894–903.
147. Nozaki K, Nakano M, Iwakami C, Fukami T, Nakajima M. RNA editing enzymes modulate the expression of hepatic CYP2B6, CYP2C8, and other cytochrome P450 isoforms. *Drug Metab Dispos* 2019;**47**:639–47.
148. Kannan B, Nagella AB, Sathia Prabhu A, Sasidharan GM, Ramesh AS, Madhugiri V. Incidence of potential drug–drug interactions in a limited and stereotyped prescription setting-comparison of two free online pharmacopoeias. *Cureus* 2016;**8**:e886.
149. Freedman MD. Drug interactions: classification and systematic approach. *Am J Ther* 1995;**2**:433–43.
150. U.S. Food and Drug Administration. *In vitro metabolism- and transporter-mediated drug–drug interaction studies guidance for industry*. 2017. Available from: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/vitro-metabolism-and-transporter-mediated-drug-drug-interaction-studies-guidance-industry>.
151. U.S. Food and Drug Administration. *Clinical drug interaction studies—study design, data analysis, and clinical implications guidance for industry*. 2017. Available from: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-drug-interaction-studies-design-data-analysis-and-clinical-implications-guidance>.
152. Min JS, Bae SK. Prediction of drug–drug interaction potential using physiologically based pharmacokinetic modeling. *Arch Pharm Res* 2017;**40**:1356–79.
153. Chen XW, Sneed KB, Pan SY, Cao C, Kanwar JR, Chew H, et al. Herb–drug interactions and mechanistic and clinical considerations. *Curr Drug Metab* 2012;**13**:640–51.
154. Tsai HH, Lin HW, Simon Pickard A, Tsai HY, Mahady GB. Evaluation of documented drug interactions and contraindications associated with herbs and dietary supplements: a systematic literature review. *Int J Clin Pract* 2012;**66**:1056–78.
155. Ge B, Zhang Z, Zuo Z. Updates on the clinical evidenced herb-warfarin interactions. *Evid Based Complement Alternat Med* 2014;**2014**:957362.
156. Lan K, Xie G, Jia W. Towards polypharmacokinetics: pharmacokinetics of multicomponent drugs and herbal medicines using a metabolomics approach. *Evid Based Complement Alternat Med* 2013;**2013**:819147.
157. Li C. Multi-compound pharmacokinetic research on Chinese herbal medicines: approach and methodology. *China J Chin Mater Med* 2017;**42**:607–17.
158. Zhang H, Bu F, Li L, Jiao Z, Ma G, Cai W, et al. Prediction of drug–drug interaction between tacrolimus and principal ingredients of wuzhi capsule in Chinese healthy volunteers using physiologically-based pharmacokinetic modelling. *Basic Clin Pharmacol* 2018;**122**:331–40.
159. Zhou H, Meibohm B. *Drug–drug interactions for therapeutic biologics*. New York: John Wiley & Sons; 2013.
160. Vugmeyster Y. Pharmacokinetics and toxicology of therapeutic proteins: advances and challenges. *World J Biol Chem* 2012;**3**:73–92.
161. Dietrich U, Dürr R, Koch J. Peptides as drugs: from screening to application. *Curr Pharm Biotechnol* 2013;**14**:501–12.
162. Ferri N, Bellosti S, Baldessin L, Boccia D, Racagni G, Corsini A. Pharmacokinetics interactions of monoclonal antibodies. *Pharmacol Res* 2016;**111**:592–9.
163. Seitz K, Zhou H. Pharmacokinetic drug–drug interaction potentials for therapeutic monoclonal antibodies: reality check. *J Clin Pharmacol* 2007;**47**:1104–18.
164. Zhuang Y, Xu Z, Frederick B, de Vries DE, Ford JA, Keen M, et al. Golimumab pharmacokinetics after repeated subcutaneous and intravenous administrations in patients with rheumatoid arthritis and the effect of concomitant methotrexate: an open-label, randomized study. *Clin Ther* 2012;**34**:77–90.
165. Baert F, Noman M, Vermeire S, Van Assche G, D’Haens G, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in crohn’s disease. *N Engl J Med* 2003;**348**:601–8.
166. Pellegrino P, Perrotta C, Clementi E, Radice S. Vaccine–drug interactions: cytokines, cytochromes, and molecular mechanisms. *Drug Saf* 2015;**38**:781–7.
167. Schmitt C, Kuhn B, Zhang X, Kivitz AJ, Grange S. Disease–drug–drug interaction involving tocilizumab and simvastatin in patients with rheumatoid arthritis. *Clin Pharmacol Ther* 2011;**89**:735–40.
168. Raaska K, Raitasuo V, Neuvonen P. Effect of influenza vaccination on serum clozapine and its main metabolite concentrations in patients with schizophrenia. *Eur J Clin Pharmacol* 2001;**57**:705–8.
169. Xu Y, Hijazi Y, Wolf A, Wu B, Sun YN, Zhu M. Physiologically based pharmacokinetic model to assess the influence of blinatumomab-mediated cytokine elevations on cytochrome P450 enzyme activity. *CPT Pharmacometrics Syst Pharmacol* 2015;**4**:507–15.
170. Jiang X, Zhuang Y, Xu Z, Wang W, Zhou H. Development of a physiologically based pharmacokinetic model to predict disease-



- mediated therapeutic protein–drug interactions: modulation of multiple cytochrome P450 enzymes by interleukin-6. *AAPS J* 2016; **18**:767–76.
171. Yang BB, Gillespie B, Smith B, Smith W, Lissmats A, Rudebeck M, et al. Pharmacokinetic and pharmacodynamic interactions between palifermin and heparin. *J Clin Pharmacol* 2015; **55**:1109–18.
  172. U.S. Food & Drug Administration. Drugs@FDA: FDA Approved Drug Products. [accessed 2018 Aug 27]. Available from: <https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm>.
  173. Genovese MC, Cohen S, Moreland L, Lium D, Robbins S, Newmark R, et al. Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate. *Arthritis Rheum* 2004; **50**: 1412–9.
  174. Gingell R, Bridges JW, Williams RT. The role of the gut flora in the metabolism of prontosil and neoprontosil in the rat. *Xenobiotica* 1971; **1**:143–56.
  175. Shu YZ, Kingston DG, Van Tassell RL, Wilkins TD. Metabolism of levamisole, an anti-colon cancer drug, by human intestinal bacteria. *Xenobiotica* 1991; **21**:737–50.
  176. Bakke OM. Degradation of dopa by intestinal microorganisms *in vitro*. *Acta Pharmacol Toxicol* 1971; **30**:115–21.
  177. Kim DH. Gut microbiota-mediated drug-antibiotic interactions. *Drug Metab Dispos* 2015; **43**:1581–9.
  178. Strong HA, Renwick AG, George CF, Liu YF, Hill MJ. The reduction of sulphinpyrazone and sulindac by intestinal bacteria. *Xenobiotica* 1987; **17**:685–96.
  179. Gingell R, Bridges JW. Intestinal azo-reduction and glucuronide conjugation of prontosil. *Xenobiotica* 1973; **3**:599–604.
  180. Zimmermann M, Zimmermann-Kogadeeva M, Wegmann R, Goodman AL. Separating host and microbiome contributions to drug pharmacokinetics and toxicity. *Science* 2019; **363**:eaat9931.
  181. Lehouritis P, Cummins J, Stanton M, Murphy CT, McCarthy FO, Reid G, et al. Local bacteria affect the efficacy of chemotherapeutic drugs. *Sci Rep* 2015; **5**:14554.
  182. Routy B, Le Chatelier E, Derosa L, Duong CP, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018; **359**:91–7.
  183. Wilkinson EM, Ilhan ZE, Herbst-Kralovetz MM. Microbiota-drug interactions: impact on metabolism and efficacy of therapeutics. *Maturitas* 2018; **112**:53–63.
  184. Fecal Microbiota Transplant (FMT) in melanoma patients. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT03341143> [accessed 2018 Sep 27].
  185. The effect of a probiotic strain on aspirin-induced GI damage. (PIP-D). Available from: <https://www.clinicaltrials.gov/ct2/show/NCT03228589> [accessed 2018 Sep 27].
  186. Prevention of irinotecan induced diarrhea by probiotics. Available from: <https://www.clinicaltrials.gov/ct2/show/NCT02819960> [accessed 2018 Sep 27].
  187. Zhang K, Chen D, Ma K, Wu X, Hao H, Jiang S. NAD(P)H: quinone oxidoreductase 1 (NQO1) as a therapeutic and diagnostic target in cancer. *J Med Chem* 2018; **61**:6983–7003.
  188. Cheng X, Liu F, Liu H, Wang G, Hao H. Enhanced glycometabolism as a mechanism of NQO1 potentiated growth of NSCLC revealed by metabolomic profiling. *Biochem Biophys Res Commun* 2018; **496**: 31–6.
  189. Staudinger JL. Disease, drug metabolism, and transporter interactions. *Pharm Res* 2013; **30**:2171–3.
  190. Liu H, Xu X, Yang Z, Deng Y, Liu X, Xie L. Impaired function and expression of P-glycoprotein in blood–brain barrier of streptozotocin-induced diabetic rats. *Brain Res* 2006; **1123**:245–52.
  191. Liu YC, Liu HY, Yang HW, Wen T, Shang Y, Liu XD, et al. Impaired expression and function of breast cancer resistance protein (Bcrp) in brain cortex of streptozotocin-induced diabetic rats. *Biochem Pharmacol* 2007; **74**:1766–72.
  192. Mei D, Li J, Liu H, Liu L, Wang X, Guo H, et al. Induction of multidrug resistance-associated protein 2 in liver, intestine and kidney of streptozotocin-induced diabetic rats. *Xenobiotica* 2012; **42**: 709–18.
  193. Shu N, Hu M, Ling Z, Liu P, Wang F, Xu P, et al. The enhanced atorvastatin hepatotoxicity in diabetic rats was partly attributed to the upregulated hepatic Cyp3a and SLCO1B1. *Sci Rep* 2016; **6**:33072.
  194. Shu N, Hu M, Liu C, Zhang M, Ling Z, Zhang J, et al. Decreased exposure of atorvastatin in diabetic rats partly due to induction of hepatic Cyp3a and Oatp2. *Xenobiotica* 2016; **46**:875–81.
  195. Xu D, Li F, Zhang M, Zhang J, Liu C, Hu MY, et al. Decreased exposure of simvastatin and simvastatin acid in a rat model of type 2 diabetes. *Acta Pharmacol Sin* 2014; **35**:1215–25.
  196. Kim YC, Lee AK, Lee JH, Lee I, Lee DC, Kim SH, et al. Pharmacokinetics of theophylline in diabetes mellitus rats: induction of CYP1A2 and CYP2E1 on 1, 3-dimethyluric acid formation. *Eur J Pharm Sci* 2005; **26**:114–23.
  197. Liu H, Liu L, Li J, Mei D, Duan R, Hu N, et al. Combined contributions of impaired hepatic CYP2C11 and intestinal breast cancer resistance protein activities and expression to increased oral glibenclamide exposure in rats with streptozotocin-induced diabetes mellitus. *Drug Metab Dispos* 2012; **40**:1104–12.
  198. Hu N, Hu M, Duan R, Liu C, Guo H, Zhang M, et al. Increased levels of fatty acids contributed to induction of hepatic CYP3A4 activity induced by diabetes—in vitro evidence from HepG2 cell and Fa2N-4 cell lines. *J Pharmacol Sci* 2014; **124**:433–44.
  199. Xie H, Sun S, Cheng X, Yan T, Zheng X, Li F, et al. Dysregulations of intestinal and colonic UDP-glucuronosyltransferases in rats with type 2 diabetes. *Drug Metab Pharmacokinet* 2013; **28**:427–34.
  200. Li P, Lu Q, Jiang W, Pei X, Sun Y, Hao H, et al. Pharmacokinetics and pharmacodynamics of rhubarb anthraquinones extract in normal and disease rats. *Biomed Pharmacother* 2017; **91**:425–35.
  201. Liu L, Miao M, Chen Y, Wang Z, Sun B, Liu X. Altered function and expression of ABC transporters at the blood–brain barrier and increased brain distribution of phenobarbital in acute liver failure mice. *Front Pharmacol* 2018; **9**:190.
  202. Wang F, Miao MX, Sun BB, Wang ZJ, Tang XG, Chen Y, et al. Acute liver failure enhances oral plasma exposure of zidovudine in rats by downregulation of hepatic UGT2B7 and intestinal P-gp. *Acta Pharmacol Sin* 2017; **38**:1554–65.
  203. Frye RF, Schneider VM, Frye CS, Feldman AM. Plasma levels of TNF- $\alpha$  and IL-6 are inversely related to cytochrome P450-dependent drug metabolism in patients with congestive heart failure. *J Card Fail* 2002; **8**:315–9.
  204. Dowling TC, Briglia AE, Fink JC, Hanes DS, Light PD, Stackiewicz L, et al. Characterization of hepatic cytochrome p4503A activity in patients with end-stage renal disease. *Clin Pharmacol Ther* 2003; **73**:427–34.
  205. Dreisbach AW, Japa S, Gebrekale AB, Mowry SE, Lertora JJ, Kamath BL, et al. Cytochrome P4502C9 activity in end-stage renal disease. *Clin Pharmacol Ther* 2003; **73**:475–7.
  206. Al Za'abi M, Shalaby A, Manoj P, Ali BH. The *in vivo* effects of adenine-induced chronic kidney disease on some renal and hepatic function and CYP450 metabolizing enzymes. *Physiol Res* 2017; **66**: 263–71.
  207. Sukkumee W, Jittisak P, Wonganan P, Wittayalertpanya S, Chariyavilaskul P, Leelahavanichkul A. The prominent impairment of liver/intestinal cytochrome P450 and intestinal drug transporters in sepsis-induced acute kidney injury over acute and chronic renal ischemia, a mouse model comparison. *Ren Fail* 2019; **41**:314–25.
  208. Lee SH, Lee SM. Suppression of hepatic cytochrome p450-mediated drug metabolism during the late stage of sepsis in rats. *Shock* 2005; **23**:144–9.
  209. Fox CS, Golden SH, Anderson C, Bray GA, Burke LE, de Boer IH, et al. Update on prevention of cardiovascular disease in adults with type 2 diabetes mellitus in light of recent evidence: a scientific

- statement from the American Heart Association and the American Diabetes Association. *Diabetes Care* 2015;**38**:1777–803.
210. Liu L, Liu XD. Alterations in function and expression of ABC transporters at blood–brain barrier under diabetes and the clinical significances. *Front Pharmacol* 2014;**5**:273.
  211. Barnett CR, Gibson GG, Wolf CR, Flatt PR, Ioannides C. Induction of cytochrome P450III and P450IV family proteins in streptozotocin-induced diabetes. *Biochem J* 1990;**268**:765–9.
  212. Hu N, Xie S, Liu L, Wang X, Pan X, Chen G, et al. Opposite effect of diabetes mellitus induced by streptozotocin on oral and intravenous pharmacokinetics of verapamil in rats. *Drug Metab Dispos* 2011;**39**:419–25.
  213. Guo Y, Hu B, Xie Y, Billiar TR, Sperry JL, Huang M, et al. Regulation of drug-metabolizing enzymes by local and systemic liver injuries. *Expert Opin Drug Metab Toxicol* 2016;**12**:245–51.
  214. Li Y, Zhang J, Xu P, Sun B, Zhong Z, Liu C, et al. Acute liver failure impairs function and expression of breast cancer-resistant protein (BCRP) at rat blood–brain barrier partly via ammonia-ROS-ERK1/2 activation. *J Neurochem* 2016;**138**:282–94.
  215. Zhang L, Chu X, Wang H, Xie H, Guo C, Cao L, et al. Dysregulations of UDP-glucuronosyltransferases in rats with valproic acid and high fat diet induced fatty liver. *Eur J Pharmacol* 2013;**721**:277–85.
  216. Zhang K, Young C, Berger J. Administrative claims analysis of the relationship between warfarin use and risk of hemorrhage including drug–drug and drug–disease interactions. *J Manag Care Pharm* 2006;**12**:640–8.
  217. Aspromonte N, Monitillo F, Puzzovivo A, Valle R, Caldarola P, Iacoviello M. Modulation of cardiac cytochrome P450 in patients with heart failure. *Expert Opin Drug Metab Toxicol* 2014;**10**:327–39.
  218. Tan FL, Moravec CS, Li J, Apperson-Hansen C, McCarthy PM, Young JB, et al. The gene expression fingerprint of human heart failure. *Proc Natl Acad Sci U S A* 2002;**99**:11387–92.
  219. El-Kadi AO, Zordoky BN. Modulation of cardiac and hepatic cytochrome P450 enzymes during heart failure. *Curr Drug Metab* 2008;**9**:122–8.
  220. Lanchote VL, Ping WC, Santos SR. Influence of renal failure on cytochrome P450 activity in hypertensive patients using a “cocktail” of antipyrine and nifedipine. *Eur J Clin Pharmacol* 1996;**50**:83–9.
  221. Guévin C, Michaud J, Naud J, Leblond FA, Pichette V. Down-regulation of hepatic cytochrome p450 in chronic renal failure: role of uremic mediators. *Br J Pharmacol* 2002;**137**:1039–46.
  222. Jacob A, Zhou M, Wu R, Wang P. The role of hepatic cytochrome P-450 in sepsis. *Int J Clin Exp Med* 2009;**2**:203–11.
  223. Park SW, Lee SM. The beneficial effect of Trolox on sepsis-induced hepatic drug metabolizing dysfunction. *Arch Pharm Res* 2004;**27**:232–8.
  224. Crawford JH, Yang S, Zhou M, Simms HH, Wang P. Down-regulation of hepatic CYP1A2 plays an important role in inflammatory responses in sepsis. *Crit Care Med* 2004;**32**:502–8.
  225. Eum HA, Yeom DH, Lee SM. Role of nitric oxide in the inhibition of liver cytochrome P450 during sepsis. *Nitric Oxide* 2006;**15**:423–31.
  226. Zhou M, Maitra SR, Wang P. The potential role of transcription factor aryl hydrocarbon receptor in downregulation of hepatic cytochrome P-450 during sepsis. *Int J Mol Med* 2008;**21**:423–8.
  227. Martin GS. Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes. *Expert Rev Anti Infect Ther* 2012;**10**:701–6.
  228. Pea F. Plasma pharmacokinetics of antimicrobial agents in critically ill patients. *Curr Clin Pharmacol* 2013;**8**:5–12.
  229. Roberts JA, Abdul-Aziz MH, Lipman J, Mouton JW, Vinks AA, Felton TW, et al. Individualised antibiotic dosing for patients who are critically ill: challenges and potential solutions. *Lancet Infect Dis* 2014;**14**:498–509.
  230. Uildemolins M, Roberts JA, Rello J, Paterson DL, Lipman J. The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. *Clin Pharmacokinet* 2011;**50**:99–110.
  231. Ito R, Takahashi T, Katano I, Ito M. Current advances in humanized mouse models. *Cell Mol Immunol* 2012;**9**:208–14.
  232. Ono C, Hsyu PH, Abbas R, Loi CM, Yamazaki S. Application of physiologically based pharmacokinetic modeling to the understanding of bosutinib pharmacokinetics: prediction of drug–drug and drug–disease interactions. *Drug Metab Dispos* 2017;**45**:390–8.
  233. Xu R, Ge W, Jiang Q. Application of physiologically based pharmacokinetic modeling to the prediction of drug–drug and drug–disease interactions for rivaroxaban. *Eur J Clin Pharmacol* 2018;**74**:755–65.
  234. Gao J, Xie W. Targeting xenobiotic receptors PXR and CAR for metabolic diseases. *Trends Pharmacol Sci* 2012;**33**:552–8.
  235. He J, Gao J, Xu M, Ren S, Stefanovic-Racic M, O’Doherty RM, et al. PXR ablation alleviates diet-induced and genetic obesity and insulin resistance in mice. *Diabetes* 2013;**62**:1876–87.
  236. He J, Nishida S, Xu M, Makishima M, Xie W. PXR prevents cholesterol gallstone disease by regulating biosynthesis and transport of bile salts. *Gastroenterology* 2011;**140**:2095–106.
  237. Zeng H, Li D, Qin X, Chen P, Tan H, Zeng X, et al. Hepatoprotective effects of *Schisandra sphenanthera* extract against lithocholic acid-induced cholestasis in male mice are associated with activation of the pregnane X receptor pathway and promotion of liver regeneration. *Drug Metab Dispos* 2016;**44**:337–42.
  238. Jiang Y, Feng D, Ma X, Fan S, Gao Y, Fu K, et al. Pregnane X receptor regulates liver size and liver cell fate by yes-associated protein activation in mice. *Hepatology* 2019;**69**:343–58.
  239. Zhai Y, Pai HV, Zhou J, Amico JA, Vollmer RR, Xie W. Activation of pregnane X receptor disrupts glucocorticoid and mineralocorticoid homeostasis. *Mol Endocrinol* 2007;**21**:138–47.
  240. Jiang M, Xie W. Role of the constitutive androstane receptor in obesity and type 2 diabetes: a case study of the endobiotic function of a xenobiotic receptor. *Drug Metab Rev* 2013;**45**:156–63.
  241. Gao J, Yan J, Xu M, Ren S, Xie W. CAR Suppresses hepatic gluconeogenesis by facilitating the ubiquitination and degradation of PGC1 $\alpha$ . *Mol Endocrinol* 2015;**29**:1558–70.
  242. He J, Lee JH, Febbraio M, Xie W. The emerging roles of fatty acid translocase/CD36 and the aryl hydrocarbon receptor in fatty liver disease. *Exp Biol Med (Maywood)* 2011;**236**:1116–21.
  243. He J, Hu B, Shi X, Weidert ER, Lu P, Xu M, et al. Activation of the aryl hydrocarbon receptor sensitizes mice to nonalcoholic steatohepatitis by deactivating mitochondrial sirtuin deacetylase Sirt3. *Mol Cell Biol* 2013;**33**:2047–55.
  244. Gong H, He J, Lee JH, Mallick E, Gao X, Li S, et al. Activation of the liver X receptor prevents lipopolysaccharide-induced lung injury. *J Biol Chem* 2009;**284**:30113–21.
  245. Zhao Z, Xu D, Li S, He B, Huang Y, Xu M, et al. Activation of liver X receptor attenuates oleic acid-induced acute respiratory distress syndrome. *Am J Pathol* 2016;**186**:2614–22.
  246. Wang H, He Q, Wang G, Xu X, Hao H. FXR modulators for enterohepatic and metabolic diseases. *Expert Opin Ther Pat* 2018;**28**:765–82.
  247. Niu Y, Xu M, Slagle BL, Huang H, Li S, Guo GL, et al. Farnesoid X receptor ablation sensitizes mice to hepatitis b virus X protein-induced hepatocarcinogenesis. *Hepatology* 2017;**65**:893–906.
  248. Chen P, Li J, Fan X, Zeng H, Deng R, Li D, et al. Oleanolic acid attenuates obstructive cholestasis in bile duct-ligated mice, possibly via activation of NRF2-MRPs and FXR antagonism. *Eur J Pharmacol* 2015;**765**:131–9.
  249. Riviere JE, Gabrielsson J, Fink M, Mochel J. Mathematical modeling and simulation in animal health. Part I: moving beyond pharmacokinetics. *J Vet Pharmacol Ther* 2016;**39**:213–23.
  250. Satoh D, Abe S, Kobayashi K, Nakajima Y, Oshimura M, Kazuki Y. Human and mouse artificial chromosome technologies for studies of pharmacokinetics and toxicokinetics. *Drug Metab Pharmacokinet* 2018;**33**:17–30.
  251. Teorell T. Kinetics of distribution of substances administered to the body. I. The extra-vascular modes of administration. *Arch Int Pharmacodyn Ther* 1937;**57**:205–25.

252. Harrison LI, Gibaldi M. Physiologically based pharmacokinetic model for digoxin disposition in dogs and its preliminary application to humans. *J Pharm Sci* 1977;**66**:1679–83.
253. Meeting of the Pharmaceutical Science and Clinical Pharmacology Advisory Committee. 2012.
254. FDA. *Physiologically based pharmacokinetic analyses-format and content guidance for industry*. 2018. Available from: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/physiologically-based-pharmacokinetic-analyses-format-and-content-guidance-industry>.
255. EMA. *Guideline on the qualification and reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation*. 2016. Available from: [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-reporting-physiologically-based-pharmacokinetic-pbpbk-modelling-simulation\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-reporting-physiologically-based-pharmacokinetic-pbpbk-modelling-simulation_en.pdf).
256. Zhao P. *Towards consistent regulatory assessment of physiologically-based pharmacokinetic modeling to support dosing recommendations*. Washington, DC: FDA; 2017.
257. Liu D, Wang K, Ma G, Xiang X, Liu J, Zhao P, et al. The value and general consideration of pharmacometric study in new drug development. *Chin J Clin Pharmacol Ther* 2018;**23**:961–73.
258. Rowland M, Peck C, Tucker G. Physiologically-based pharmacokinetics in drug development and regulatory science. *Annu Rev Pharmacol Toxicol* 2011;**51**:45–73.
259. Hsueh CH, Hsu V, Pan Y, Zhao P. Predictive performance of physiologically-based pharmacokinetic models in predicting drug–drug interactions involving enzyme modulation. *Clin Pharmacokinet* 2018;**57**:1337–46.
260. Zhang L, Huang SM, Lesko LJ. Transporter-mediated drug–drug interactions. *Clin Pharmacol Ther* 2011;**89**:481–4.
261. Aghazadeh-Habashi A, Asghar W, Jamali F. Drug–disease interaction: effect of inflammation and nonsteroidal anti-inflammatory drugs on cytochrome p450 metabolites of arachidonic acid. *J Pharm Sci* 2018;**107**:756–63.
262. Tan ML, Yoshida K, Zhao P, Zhang L, Nolin TD, Piquette-Miller M, et al. Effect of chronic kidney disease on nonrenal elimination pathways: a systematic assessment of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and OATP. *Clin Pharmacol Ther* 2018;**103**:854–67.
263. Yoshida K, Budha N, Jin JY. Impact of physiologically based pharmacokinetic models on regulatory reviews and product labels: frequent utilization in the field of oncology. *Clin Pharmacol Ther* 2017;**101**:597–602.
264. Wagner C, Pan Y, Hsu V, Grillo JA, Zhang L, Reynolds KS, et al. Predicting the effect of cytochrome P450 inhibitors on substrate drugs: analysis of physiologically based pharmacokinetic modeling submissions to the US Food and Drug Administration. *Clin Pharmacokinet* 2015;**54**:117–27.
265. Wagner C, Pan Y, Hsu V, Sinha V, Zhao P. Predicting the effect of CYP3A inducers on the pharmacokinetics of substrate drugs using physiologically based pharmacokinetic (PBPK) modeling: an analysis of PBPK submissions to the US FDA. *Clin Pharmacokinet* 2016;**55**:475–83.
266. Wagner C, Zhao P, Pan Y, Hsu V, Grillo J, Huang SM, et al. Application of physiologically based pharmacokinetic (PBPK) modeling to support dose selection: report of an FDA public workshop on PBPK. *CPT Pharmacometrics Syst Pharmacol* 2015;**4**:226–30.
267. Guo Y, Chu X, Parrott NJ, Brouwer KL, Hsu V, Nagar S, et al. Advancing predictions of tissue and intracellular drug concentrations using *in vitro*, imaging and physiologically based pharmacokinetic modeling approaches. *Clin Pharmacol Ther* 2018;**104**:865–89.
268. Pan Y, Hsu V, Grimstein M, Zhang L, Arya V, Sinha V, et al. The application of physiologically based pharmacokinetic modeling to predict the role of drug transporters: scientific and regulatory perspectives. *J Clin Pharmacol* 2016;**56**(Suppl 7):S122–31.
269. Nguyen TV, Ukairo O, Khetani SR, McVay M, Kanchagar C, Seghezzi W, et al. Establishment of a hepatocyte-kupffer cell coculture model for assessment of proinflammatory cytokine effects on metabolizing enzymes and drug transporters. *Drug Metab Dispos* 2015;**43**:774–85.
270. Yoshida K, Sun B, Zhang L, Zhao P, Abernethy DR, Nolin TD, et al. Systematic and quantitative assessment of the effect of chronic kidney disease on CYP2D6 and CYP3A4/5. *Clin Pharmacol Ther* 2016;**100**:75–87.
271. Feng B, Varma MV. Evaluation and quantitative prediction of renal transporter-mediated drug–drug interactions. *J Clin Pharmacol* 2016;**56**(Suppl 7):S110–21.
272. Hsu V, de L T Vieira M, Zhao P, Zhang L, Zheng JH, Nordmark A, et al. Towards quantitation of the effects of renal impairment and probenecid inhibition on kidney uptake and efflux transporters, using physiologically based pharmacokinetic modelling and simulations. *Clin Pharmacokinet* 2014;**53**:283–93.
273. Edginton AN, Willmann S. Physiology-based simulations of a pathological condition: prediction of pharmacokinetics in patients with liver cirrhosis. *Clin Pharmacokinet* 2008;**47**:743–52.
274. Schlender JF, Meyer M, Thelen K, Krauss M, Willmann S, Eissing T, et al. Development of a whole-body physiologically based pharmacokinetic approach to assess the pharmacokinetics of drugs in elderly individuals. *Clin Pharmacokinet* 2016;**55**:1573–89.
275. Yellepeddi V, Rower J, Liu X, Kumar S, Rashid J, Sherwin CM. State-of-the-art review on physiologically based pharmacokinetic modeling in pediatric drug development. *Clin Pharmacokinet* 2019;**58**:1–13.
276. Abduljalil K, Jamei M, Johnson TN. Fetal physiologically based pharmacokinetic models: systems information on the growth and composition of fetal organs. *Clin Pharmacokinet* 2019;**58**:235–62.
277. Xia B, Heimbach T, Gollen R, Nanavati C, He H. A simplified PBPK modeling approach for prediction of pharmacokinetics of four primarily renally excreted and CYP3A metabolized compounds during pregnancy. *AAPS J* 2013;**15**:1012–24.
278. Lin L, Wong H. Predicting Oral Drug Absorption: mini review on physiologically-based pharmacokinetic models. *Pharmaceutics* 2017;**9**:41.
279. Mitra A. Maximizing the role of physiologically based oral absorption modeling in generic drug development. *Clin Pharmacol Ther* 2019;**105**:307–9.
280. Suarez-Sharp S, Cohen M, Kesisoglou F, Abend A, Marroum P, Delvadia P, et al. Applications of clinically relevant dissolution testing: workshop summary report. *AAPS J* 2018;**20**:93.
281. Tsume Y, Patel S, Fotaki N, Bergström C, Amidon GL, Brasseur JG, et al. *In vivo* predictive dissolution and simulation workshop report: facilitating the development of oral drug formulation and the prediction of oral bioperformance. *AAPS J* 2018;**20**:100.
282. Fang L, Kim MJ, Li Z, Wang Y, Diliberti CE, Au J, et al. Model-informed drug development and review for generic products: summary of FDA public workshop. *Clin Pharmacol Ther* 2018;**104**:27–30.
283. Kesisoglou F, Mitra A. Application of absorption modeling in rational design of drug product under quality-by-design paradigm. *AAPS J* 2015;**17**:1224–36.
284. Babiskin AH, Zhang X. Application of physiologically based absorption modeling for amphetamine salts drug products in generic drug evaluation. *J Pharm Sci* 2015;**104**:3170–82.
285. Margolskee A, Darwich AS, Pepin X, Aarons L, Galetin A, Rostami-Hodjegan A, et al. IMI – oral biopharmaceutics tools project-evaluation of bottom-up PBPK prediction success part 2: an introduction to the simulation exercise and overview of results. *Eur J Pharm Sci* 2017;**96**:610–25.
286. Vuppugalla R, Marathe P, He H, Jones RD, Yates JW, Jones HM, et al. PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 4: prediction of plasma concentration-time profiles in human from *in vivo* preclinical data by using the Wajima approach. *J Pharm Sci* 2011;**100**:4111–26.
287. Cao Y, Jusko WJ. Applications of minimal physiologically-based pharmacokinetic models. *J Pharmacokinet Pharmacodyn* 2012;**39**:711–23.



288. Cao Y, Jusko WJ. Survey of monoclonal antibody disposition in man utilizing a minimal physiologically-based pharmacokinetic model. *J Pharmacokinet Pharmacodyn* 2014;**41**:571–80.
289. Wong H, Chow TW. Physiologically based pharmacokinetic modeling of therapeutic proteins. *J Pharm Sci* 2017;**106**:2270–5.
290. Niederalt C, Kuepfer L, Solodenko J, Eissing T, Siegmund HU, Block M, et al. A generic whole body physiologically based pharmacokinetic model for therapeutic proteins in PK-Sim. *J Pharmacokinet Pharmacodyn* 2018;**45**:235–57.
291. Liu D, Song H, Song L, Liu Y, Cao Y, Jiang J, et al. A unified strategy in selection of the best allometric scaling methods to predict human clearance based on drug disposition pathway. *Xenobiotica* 2016;**46**:1105–11.
292. Liu D, Ma X, Liu Y, Zhou H, Shi C, Wu F, et al. Quantitative prediction of human pharmacokinetics and pharmacodynamics of imigluptin, a novel DPP-4 inhibitor, using allometric scaling, IVIVE and PK/PD modeling methods. *Eur J Pharm Sci* 2016;**89**:73–82.
293. Song L, Zhang Y, Jiang J, Ren S, Chen L, Liu D, et al. Development of a Physiologically based pharmacokinetic model for sinoglatin, a first-in-class glucokinase activator, by integrating allometric scaling, *in vitro* to *in vivo* exploration and steady-state concentration-mean residence time methods: mechanistic understanding of its pharmacokinetics. *Clin Pharmacokinet* 2018;**57**:1307–23.
294. Rose RH, Neuhoﬀ S, Abduljalil K, Chetty M, Rostami-Hodjegan A, Jamei M. Application of a physiologically based pharmacokinetic model to predict *OATP1B1*-related variability in pharmacodynamics of rosuvastatin. *CPT Pharmacometrics Syst Pharmacol* 2014;**3**:e124.
295. Chen Y, Zhao K, Liu F, Li Y, Zhong Z, Hong S, et al. Predicting antitumor effect of deoxypodophyllotoxin in NCI-H460 tumor-bearing mice on the basis of *in vitro* pharmacodynamics and a physiologically based pharmacokinetic-pharmacodynamic model. *Drug Metab Dispos* 2018;**46**:897–907.
296. Feng S, Shi J, Parrott N, Hu P, Weber C, Martin-Facklam M, et al. Combining ‘bottom-up’ and ‘top-down’ methods to assess ethnic difference in clearance: bitopertin as an example. *Clin Pharmacokinet* 2016;**55**:823–32.
297. Jorga K, Chavanne C, Frey N, Lave T, Lukacova V, Parrott N, et al. Bottom-up meets top-down: complementary physiologically based pharmacokinetic and population pharmacokinetic modeling for regulatory approval of a dosing algorithm of valganciclovir in very young children. *Clin Pharmacol Ther* 2016;**100**:761–9.
298. Gufford BT, Barr JT, González-Pérez V, Layton ME, White Jr JR, Oberlies NH, et al. Quantitative prediction and clinical evaluation of an unexplored herb–drug interaction mechanism in healthy volunteers. *CPT Pharmacometrics Syst Pharmacol* 2015;**4**:701–10.
299. Capecchi MR. Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. *Nat Rev Genet* 2005;**6**:507–12.
300. Gaj T, Gersbach CA, Barbas III CF, ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol* 2013;**31**:397–405.
301. Qiu Z, Liu M, Chen Z, Shao Y, Pan H, Wei G, et al. High-efficiency and heritable gene targeting in mouse by transcription activator-like effector nucleases. *Nucleic Acids Res* 2013;**41**:e120.
302. Diliberto JJ, Burgin D, Birnbaum LS. Role of CYP1A2 in hepatic sequestration of dioxin: studies using CYP1A2 knock-out mice. *Biochem Biophys Res Commun* 1997;**236**:431–3.
303. Lu Y, Wu D, Wang X, Ward SC, Cederbaum AI. Chronic alcohol-induced liver injury and oxidant stress are decreased in cytochrome P4502E1 knockout mice and restored in humanized cytochrome P4502E1 knock-in mice. *Free Radic Biol Med* 2010;**49**:1406–16.
304. Scheer N, Kapelyukh Y, Chatham L, Rode A, Buechel S, Wolf CR. Generation and characterization of novel cytochrome P450 Cyp2c gene cluster knockout and CYP2C9 humanized mouse lines. *Mol Pharmacol* 2012;**82**:1022–9.
305. van Waterschoot RA, van Herwaarden AE, Lagas JS, Sparidans RW, Wagenaar E, van der Kruijsen CM, et al. Midazolam metabolism in cytochrome P450 3A knockout mice can be attributed to up-regulated CYP2C enzymes. *Mol Pharmacol* 2008;**73**:1029–36.
306. Dragin N, Uno S, Wang B, Dalton TP, Nebert DW. Generation of ‘humanized’ hCYP1A1\_1A2\_Cyp1a1/1a2<sup>-/-</sup> mouse line. *Biochem Biophys Res Commun* 2007;**359**:635–42.
307. Löfgren S, Baldwin RM, Hiratsuka M, Lindqvist A, Carlberg A, Sim SC, et al. Generation of mice transgenic for human CYP2C18 and CYP2C19: characterization of the sexually dimorphic gene and enzyme expression. *Drug Metab Dispos* 2008;**36**:955–62.
308. Hasegawa M, Kapelyukh Y, Tahara H, Seibler J, Rode A, Krueger S, et al. Quantitative prediction of human pregnane X receptor and cytochrome P450 3A4 mediated drug–drug interaction in a novel multiple humanized mouse line. *Mol Pharmacol* 2011;**80**:518–28.
309. Scheer N, Kapelyukh Y, McEwan J, Beuger V, Stanley LA, Rode A, et al. Modeling human cytochrome P450 2D6 metabolism and drug–drug interaction by a novel panel of knockout and humanized mouse lines. *Mol Pharmacol* 2012;**81**:63–72.
310. Liu Z, Li L, Wu H, Hu J, Ma J, Zhang QY, et al. Characterization of CYP2B6 in a CYP2B6-humanized mouse model: inducibility in the liver by phenobarbital and dexamethasone and role in nicotine metabolism *in vivo*. *Drug Metab Dispos* 2015;**43**:208–16.
311. Zanger UM, Klein K, Thomas M, Rieger JK, Tremmel R, Kandel BA, et al. Genetics, epigenetics, and regulation of drug-metabolizing cytochrome p450 enzymes. *Clin Pharmacol Ther* 2014;**95**:258–61.
312. Aitman TJ, Critser JK, Cuppen E, Dominiczak A, Fernandez-Suarez XM, Flint J, et al. Progress and prospects in rat genetics: a community view. *Nat Genet* 2008;**40**:516–22.
313. Yoshimi K, Kunihiro Y, Kaneko T, Nagahora H, Voigt B, Mashimo T. ssODN-mediated knock-in with CRISPR-Cas for large genomic regions in zygotes. *Nat Commun* 2016;**7**:10431.
314. Wang X, Tang Y, Lu J, Shao Y, Qin X, Li Y, et al. Characterization of novel cytochrome P450 2E1 knockout rat model generated by CRISPR/Cas9. *Biochem Pharmacol* 2016;**105**:80–90.
315. Lu J, Shao Y, Qin X, Liu D, Chen A, Li D, et al. CRISPR knockout rat cytochrome P450 3A1/2 model for advancing drug metabolism and pharmacokinetics research. *Sci Rep* 2017;**7**:42922.
316. Lu J, Liu M, Wang X. Gene targeting on Cyp2c locus in rats using the CRISPR/Cas9 system. In: Wang X, editor. *CRISPR: advances in research and applications*. New York: NOVA Science Publishers, Inc; 2017. p. 95–111.
317. Wei Y, Yang L, Zhang X, Sui D, Wang C, Wang K, et al. Generation and characterization of a CYP2C11-null rat model by using the CRISPR/Cas9 method. *Drug Metab Dispos* 2018;**46**:525–31.
318. Kumar R, Mota LC, Litoff EJ, Rooney JP, Boswell WT, Courter E, et al. Compensatory changes in CYP expression in three different toxicology mouse models: cAR-null, Cyp3a-null, and Cyp2b9/10/13-null mice. *PLoS One* 2017;**12**:e0174355.
319. Liang C, Zhao J, Lu J, Zhang Y, Ma X, Shang X, et al. Development and characterization of MDR1 (*Mdr1a/b*) CRISPR/Cas9 knockout rat model. *Drug Metab Dispos* 2019;**47**:71–9.
320. Qin X, Lu J, Wang P, Xu P, Liu M, Wang X. Cytochrome P450 3A selectively affects the pharmacokinetic interaction between erlotinib and docetaxel in rats. *Biochem Pharmacol* 2017;**143**:129–39.
321. Schneider KJ, DeCaprio AP. Covalent thiol adducts arising from reactive intermediates of cocaine biotransformation. *Chem Res Toxicol* 2013;**26**:1755–64.
322. Lai WG, Farah N, Moniz GA, Wong YN. A Baeyer-Villiger oxidation specifically catalyzed by human flavin-containing monooxygenase 5. *Drug Metab Dispos* 2011;**39**:61–70.
323. Meng J, Zhong D, Li L, Yuan Z, Yuan H, Xie C, et al. Metabolism of MRX-I, a novel antibacterial oxazolidinone, in humans: the oxidative ring opening of 2, 3-dihydropyridin-4-one catalyzed by non-P450 enzymes. *Drug Metab Dispos* 2015;**43**:646–59.

324. Aigrain L, Pompon D, Truan G. Role of the interface between the FMN and FAD domains in the control of redox potential and electronic transfer of NADPH-cytochrome P450 reductase. *Biochem J* 2011;**435**:197–206.
325. Hou X, Zhou J, Yu S, Zhou L, Zhang Y, Zhong D, et al. Differences in the *in vivo* and *in vitro* metabolism of imrecoxib in humans: formation of the rate-limiting aldehyde intermediate. *Drug Metab Dispos* 2018;**46**:1320–8.
326. Fukami T, Yokoi T. The emerging role of human esterases. *Drug Metab Pharmacokinet* 2012;**27**:466–77.
327. Kurokawa T, Fukami T, Yoshida T, Nakajima M. Arylacetamide deacetylase is responsible for activation of prasugrel in human and dog. *Drug Metab Dispos* 2016;**44**:409–16.
328. Jiang J, Chen X, Zhong D. Arylacetamide deacetylase is involved in vicagrel bioactivation in humans. *Front Pharmacol* 2017;**8**:846.
329. Liu C, Chen XY, Zhong D. Metabolism and pharmacokinetics of vica-grel, a novel thienopyridine P2y<sub>12</sub> inhibitor, compared with clopidogrel in healthy Chinese subjects. *Drug Metab Pharmacokinet* 2017;**32**: S93–4.
330. Fleming FF, Yao L, Ravikumar PC, Funk L, Shook BC. Nitrile-containing pharmaceuticals: efficacious roles of the nitrile pharmacophore. *J Med Chem* 2010;**53**:7902–17.
331. Kong F, Pang X, Zhao J, Deng P, Zheng M, Zhong D, et al. Hydrolytic metabolism of cyanopyrrolidine DPP-4 inhibitors mediated by dipeptidyl peptidases. *Drug Metab Dispos* 2019;**47**:238–48.
332. Gao R, Li L, Xie C, Diao X, Zhong D, Chen X. Metabolism and pharmacokinetics of morinidazole in humans: identification of diastereoisomeric morpholine N<sup>+</sup>-glucuronides catalyzed by UDP glucuronosyltransferase 1A9. *Drug Metab Dispos* 2012;**40**:556–67.
333. Zhou D, Guo J, Linnenbach AJ, Booth-Genthe CL, Grimm SW. Role of human UGT2B10 in *N*-glucuronidation of tricyclic antidepressants, amitriptyline, imipramine, clomipramine, and trimipramine. *Drug Metab Dispos* 2010;**38**:863–70.
334. Kaivosaaari S, Finel M, Koskinen M. *N*-Glucuronidation of drugs and other xenobiotics by human and animal UDP-glucuronosyltransferases. *Xenobiotica* 2011;**41**:652–69.
335. Xue P, Liu D, Wang J, Zhang N, Zhou J, Li L, et al. Redox-sensitive citronellol-cabazitaxel conjugate: maintained *in vitro* cytotoxicity and self-assembled as multifunctional nanomedicine. *Bioconjug Chem* 2016;**27**:1360–72.
336. Yamaguchi T, Nakajima Y, Nakamura Y. Possible mechanism for species difference on the toxicity of pivalic acid between dogs and rats. *Toxicol Appl Pharm* 2006;**214**:61–8.
337. Qiu F, Cui L, Chen L, Sun J, Yao X. Two novel creatinine adducts of andrographolide in human urine. *Xenobiotica* 2012;**42**:911–6.
338. von Richter O, Massimini G, Scheible H, Udvaros I, John A. Pimasertib, a selective oral MEK1/2 inhibitor: absolute bioavailability, mass balance, elimination route, and metabolite profile in cancer patients. *Br J Clin Pharmacol* 2016;**82**: 1498–508.
339. Yin W, Doss GA, Stearns RA, Kumar S. *N*-Acetylation of the glutamate residue of intact glutathione conjugates in rats: a novel pathway for the metabolic processing of thiol adducts of xenobiotics. *Drug Metab Dispos* 2004;**32**:43–8.
340. Savage RE, Tyler AN, Miao XS, Chan TC. Identification of a novel glucosylsulfate conjugate as a metabolite of 3,4-dihydro-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5,6-dione (ARQ 501, beta-lapachone) in mammals. *Drug Metab Dispos* 2008;**36**:753–8.